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Poster Presentations

Track 1: Infectious diseases

1.1 Non malarial febrile illness: challenges in epidemiology, diagnosis and treatment

P.1.1.001 (B)

The treatment of non-malaria febrile illness in Papua New Guinea: findings from cross sectional and longitudinal studies of health worker practice

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INTRODUCTION Papua New Guinea (PNG), a malaria endemic nation of approximately seven million people, introduced a revised national malaria treatment protocol (NMTP) in late 2011. Consistent with current WHO guidelines, the new NMTP stipulates that all fever or suspected malaria cases be tested for malaria infection by microscopy or rapid diagnostic test (RDT) and that anti-malarials should only be prescribed upon confirmation of malaria parasitaemia. This represents a substantial change in recommended malaria case management as, prior to the new NMTP, the presumptive treatment of fever patients with anti-malarials was a near universal practice. This paper presents findings from the first studies to examine how PNG health workers are managing non-malaria febrile patients post implementation of the revised NMTP.
METHOD Data were collected via a countrywide cross-sectional survey of febrile case management patients (n = 556) from 88 randomly selected health facilities in 2012 and from longitudinal monitoring of febrile patients (n = 2000+) in seven health facility surveillance sites across the country.
RESULTS AND CONCLUSION At the time of writing this abstract, the aforementioned datasets were undergoing final data cleaning prior to analysis. Planned analyses will include a descriptive overview of current diagnostic and treatment practices as well as multiple regression analyses incorporating patient, health worker and health service variables to identify potential predictors of health worker practice. It is anticipated that the resulting data will provide a detailed picture of health worker diagnostic and treatment practices pertaining to non-malaria febrile patients in PNG and should inform possible training or other health worker support needs.

P.1.1.002 (B)

Trusting malaria test results: fever and young age predicts inappropriate malaria treatment in Uganda

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INTRODUCTION Health care workers (HCW) continue to presumptively treat fever cases as malaria. This results in over-prescription of malaria treatment, leaving other causes of fever untreated. We conducted a secondary analysis of data from the Integrated Infectious Disease Capacity Building Evaluation (IDCAP), focusing on a subgroup of patients with a history of fever.
MATERIAL AND METHODS We analyzed data collected from 36 health centers from November 2009 to December 2010 using a revised Uganda Ministry of Health Outpatient Medical Form and compared pediatric (under 5 years of age) and older fever cases. Given the data were originally collected for evaluation of training interventions, we controlled for the impact of the interventions.
RESULTS Data from 753 074 (27.5% pediatric) outpatient visits was analyzed. Fever was reported in 333 371 (44%) visits of which 128 205 (38%) received a malaria blood test and 68 277 (42%) tested negative. After controlling for the effects of the intervention, pediatric fever cases were more likely than older cases to receive a malaria test (43% vs. 35%, RR = 1.20, 99%CI: 1.06, 1.36; $P < 0.001$). Pediatric fever cases with negative test results were also more likely to be diagnosed with malaria (46% vs. 34%, RR = 1.32, 99%CI: 1.18, 1.48; $P < 0.001$) and prescribed antimalarial treatment (55% vs. 40%, RR = 1.35, 99%CI: 1.19, 1.53; $P < 0.001$).
CONCLUSIONS While inappropriate treatment of smear negative fever cases was high among both age groups, pediatric fever cases were more likely than older cases to be inappropriately treated for malaria. HCW may be less likely trust results of malaria tests among pediatric fever cases due to the higher risk of mortality from malaria among children. More research is needed to better understand the HCW decision-making process for treatment of fever cases testing negative for malaria among both pediatric and older cases.

P.1.1.003 (B)

Treating febrile children in sub-Saharan Africa: evidence from national household surveys in Madagascar, Nigeria and Uganda

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BRIEF INTRODUCTION Appropriate case management is a key control target for all childhood illnesses. The focus of indicators collected from population-based surveys is often restricted by illness, with little examination of polypharmacy or the use by caregivers of multiple sources of advice and treatment. Understanding how caregivers respond to an illness episode in its entirety merits further investigation.
MATERIAL AND METHODS Nationally-representative household surveys focused on treatment-seeking behaviour for

fever among children under five were conducted in 2012 in Madagascar, Nigeria and Uganda as part of the ACTwatch program. Detailed information on treatment-seeking behaviour was collected, including where advice and treatment was sought, and diagnostic services and medicines received at each source. Unlike standard population-based surveys the ACTwatch questionnaire uses an audit mechanism that enables brand and active ingredient details to be recorded from available medicine packages, reducing the likelihood of recall bias. Treatment indicators are tabulated by country, and cross-tabulated by urban and rural location.
RESULTS In all three countries caregivers most commonly first sought treatment for a child's fever at home (Madagascar 44%, Nigeria 48%, Uganda 61%). Treatment was first sought from the informal private sector for 20% of fevers in Madagascar. Additional source indicators will present the number of external sources visited and the source mix. Results will be presented on the proportion of children receiving single and multiple medicines and the distribution of medicine types received from each source. For example, in Uganda 16% of children received both an antimalarial and antibiotic during their fever episode.
CONCLUSIONS Treatment-seeking for childhood illness is a dynamic process, frequently involving multiple treatments that are often sourced from several providers. This complex picture is masked by the standard approach to reporting population-based indicators. The information presented in this work has the potential to inform programming and health promotion campaigns.

P.1.1.004 (B)

Prevalence of malaria diagnosis in infants below 6 months of age presenting with fever at rural health facilities in Uganda

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BACKGROUND Malaria continues to be among the leading causes of mortality and morbidity due to infectious diseases especially among children under five. Recent studies have indicated a higher prevalence of malaria in young infants than previously estimated. Evidence to inform treatment recommendations for this age group is lacking due to the limited number of clinical trials and sub analysis of studies focusing on this age group. Updated information on prevalence of clinical malaria among young infants is needed to inform and draw attention of research efforts for tools and approaches for malaria control in this age group.
METHODS A cross-sectional survey of records on outpatient care of all children aged less than five with a fever attending outpatient care between January and October 2010 at 36 rural sub district hospitals in Uganda was conducted. Prevalence of clinical malaria defined as proportion of children with a fever with positive results for a malaria diagnostic test as main outcome. Age group comparing young infants (0–5 months) to older infants (6–11 months) was the main explanatory variable.
RESULTS Overall prevalence of clinical malaria among children under five was 50%. Twenty three percent of malaria cases were aged <12 months; 32% were aged 12–23 months and 45% were aged 24–60 months. Prevalence of clinical malaria was 39.9% (AOR 0.73, 95%CI 0.682–0.791, $P < 0.0000$) in young infants (0–5 months) compared to 41.1% in older infants (6–11 months).
CONCLUSIONS The findings show that prevalence of clinical malaria in Uganda measured among infants with fever attending rural outpatient care is higher than previously estimated. More research is needed to review appropriateness of current malaria treatment regimes for this age group and their implications on health outcomes of young infants diagnosed with malaria.

1.2 Control of Neglected Zoonotic Diseases using a One Health approach

P.1.2.001 (A)

Northern Slovakia – highly endemic area of alveolar echinococcosis

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In Slovakia, *Echinococcus multilocularis*, causative agent of human alveolar echinococcosis, was detected in red foxes in 1999 for the first time. Following long-term surveillance of tapeworm occurrence in red foxes revealed the existence of highly endemic areas in northern parts of the country, with overall prevalence rate of 41.6%. In some localities of Pre¹ov and ®ilina regions the prevalence reaching as much as 60% was recorded. Between 2000 and 2012 in total 24 human cases of alveolar echinococcosis was detected and only three of them did not came from endemic localities of northern Slovakia. Remarkable is the occurrence of the disease in eight persons <35 years, including three patients younger than 20 years. Occurrence of *E. multilocularis* in red foxes in whole territory of the country, together with increasing incidence of alveolar echinococcosis in humans and especially in young people indicates the high infectious pressure of northern Slovakia environment and the threat for both, humans and animals. The research has drawn attention to necessity of targeted surveillance and engagement of specialist, veterinarians, zoologists and ecologists, for effective managements of the diseases for effective managements of the diseases prevention.

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P.1.2.002 (A)

Assessment of the social burden of *Taenia solium* Cysticercosis in Angónia District, Mozambique

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INTRODUCTION *Taenia solium* cysticercosis is a zoonosis of both public health and agricultural importance in many low-income countries. This study aimed at estimating the societal burden of *T. solium* cysticercosis in Angónia district, Mozambique, an area highly endemic for the disease.
MATERIALS AND METHODS Epidemiological data on human and porcine cysticercosis were collected from 2008 to 2009 in Angónia district, and made available for burden assessment. Study subjects were 1723 persons and 661 pigs. Methods included a questionnaire survey, Ag-ELISA detection of human and porcine cysticercosis and human brain computer tomography. All data were compiled in the software for statistical analysis 'R'. To estimate the DALYs lost due to neurocysticercosis – associated epilepsy and headache a DALY

calculator was used. To estimate the total costs a cost analysis model was used.
RESULTS Approximately 5% and 0.8% of the total population of Angónia district was estimated to suffer from NCC-associated epilepsy and headache, respectively. Around two thirds of the diseased population never received any treatment. The estimates were based on reported prevalence of epilepsy and headache of 15.6% and 30.9%, respectively. Among people with reported epilepsy, 42.5% had NCC. The number of pigs diagnosed with cysticercosis corresponded to 35% of the total pig population. The estimated average number of DALYs lost due to NCC-associated epilepsy and headache was 12.1 per thousand persons per year. The total annual costs due to *T. solium* cysticercosis were estimated at 1.3 million Euro of which 87% were costs linked to human cysticercosis and 13% were due to pig production losses. The annual monetary burden per case of NCC-associated epilepsy amounted at 51.0 Euro.
CONCLUSIONS Twelve DALYs per thousand persons per year and a cost of more than one million Euro per year makes *T. solium* cysticercosis a serious public health and agricultural threat for Angónia district.

P.1.2.004 (A)

Ticks are underestimated zoonotic vectors in Egypt

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General speaking, Ticks either soft or hard are prevalent worldwide particularly in farm animals rearing countries. In Egypt where there are many rural and urban areas, so many genera and species of ticks are encountered. The Egyptian veterinary and agricultural authorities deal with ticks from economic point of view. But from medical point of view, ticks have specific role in transmission of zoonotic infectious diseases as well as their saliva causes tick paralysis. Recently, CCHF and babesiosis as well as infantile tick paralysis were identified as emerging diseases disaster. On the other hand, Lyme disease or Lyme borreliosis is the most common worldwide tick-borne disease was encountered in Egypt since a long time. No doubt junior physician and of course the native in rural areas are not aware of ticks and their medical disaster role.
KEY WORDS Egypt, Crimean Congo Hemorrhagic Fever, Babesiosis, Ticks.

P.1.2.005 (A)

Added value of the 'One Health' perspective – the SLIPP angle: securing rural livelihoods through improved smallholder pig production in Mozambique and Tanzania

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Pigs represent an important source of income and protein in rural communities of Eastern and Southern Africa (ESA). However, smallholder pig production systems are generally severely constrained by poor management of pigs and associated diseases such as *Taenia solium* cysticercosis (TSC) and African swine fever (ASF). As a consequence pig performance, health and welfare are often compromised just as public health is threatened due to TSC affected pork. One of the main challenges in improving the situation is the farmer's superstitions and lack of knowledge of disease pathways and basic requirements of pigs. The current project therefore uses a multifactorial 'One Health' approach to address the complexity of changing not only management practices, animal and human health but also perceptions and prejudices. This involves awareness generation to improve overall public knowledge and community based research to document effect and feasibility at village level, in order to persuade farmers and other stakeholders to adapt solutions long-term. Small-scale testing of management tools have been carried out through student projects and the results and experiences combined in optimized pig production models tailored to conditions in Mozambique and Tanzania. The production model focuses on confinement of pigs as the key entry point for controlling TSC and ASF. To alleviate some of the problems associated with confinement, farmers and communities are provided with education on pen construction, disease control, breeding and how to provide suitable feeds and water. The production models are then assessed for effectiveness, efficacy and acceptability among smallholder pig farmers in selected communities. The impact on TSC, ASF, ecto- and endoparasites is also monitored and farmer livelihood is assessed before and after introduction of the production models. Once the project is completed in 2015 it is believed to have helped increase pig productivity, food security and human health in ESA.

P.1.2.006 (A)

The Rift Valley fever: is it a neglected infectious disease in Egypt?

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The Rift Valley fever (RVF) is a neglected, emerging, mosquito-borne disease with severe negative impact on human and animal health and economy. RVF is caused by RVF virus of the family of Bunyaviridae, genus Phlebovirus. RVF is an acute, febrile disease affecting humans and a wide range of animals. The virus is transmitted through bites from mosquitoes and exposure to viremic blood, body fluids, or contact with tissues of infected animals or by inhaling natural virus aerosols, also possibly by consumption of infected unpasteurized milk. The RVF-virus replicate at the site introduction and in local lymphatic followed by viremia and spread to other organs as the liver and central nervous system, causing the hepatic necrosis and eosinophilia cytoplasmic degeneration. The main signs and symptoms are fever, headache, myalgia, arthralgia, photophobia, bradycardia, conjunctivitis and flushing face. Main complications include jaundice, hemorrhagic, meningoencephalitis and retinal lesions.
KEY WORDS Egypt, Rift Valley Fever, Mosquitoes, Re-Emergence.

P.1.2.007 (A)

Healthy pigs for healthy people. A cysticercosis advocacy information tool

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Porcine cysticercosis is an infection of pigs caused by the larval stage of *Taenia solium*, a tapeworm that causes taeniosis in

humans. The disease is common in developing countries and is a serious public health risk. Cysticercosis implicates significant economic losses, both in pig production and its impact on human health causing severe headaches and seizures. Cysticercosis control strategies in developing countries have been limited by a lack of available intervention tools and poor socioeconomic and sanitary conditions. Consequently, the intervention strategies to prevent and control cysticercosis must be on health education engaging the communities and creating public awareness. Enhancing basic conditions such as hygiene has an important effect on reducing the risk of transmission. In addition some very simple but effective changes in people's day-to-day practices such as use of latrines and keeping pigs in pens would stop the life cycle of the disease and considerably reduce the risk of cysticercosis transmission. The need for political will and resources are basic requirements in order to control not only cysticercosis but also other endemic zoonotic diseases. ICONZ and ADVANZ are two One Health neglected zoonotic diseases projects, funded by the European Commission through its 7th framework program. Part of University of Copenhagen's tasks in these projects is to develop an improved advocacy tool for teaching about cysticercosis, including information on how to diagnose and treat the disease in both pigs and humans, its impact on people's livelihood, and possible control and intervention strategies The advocacy tool will be developed as a USB flash drive, with information targeted at three levels: knowledge relevant to the laymen in the villages, information for supporting practitioners; MD's in health centres, veterinary and agricultural extension officers and pig traders. Furthermore there will be a policy brief aimed at the key decision makers at ministry level.

P.1.2.008 (A)

Filariasis and blackflies (Diptera: Simuliidae) in Europe: Portugal

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INTRODUCTION Animal onchocerciasis may be caused by different species of *Onchocerca* (Spirurida: Onchocercidae). In Portugal, *Onchocerca guttorosa* (Neumann, 1910) and *Onchocerca* sp.were identified in cattle and equine species by Roque & Mendonça (1982), Mendonça & Roque (1982), Grácio (1991) and Grácio & Grácio (2012), and *O. lupi* Rodojana, 1967 was responsible for canine ocular onchocersiasis (Faìsca et al., 2010). Alexandre et al. (2006) reported ocular disease by *Onchocerca* sp. in a dog from Algarve. Simulium (Wilhelmia) pseudequinum Séguy, 1921 was incriminated vector of *Onchocerca* sp. and S. (Simulium) nitidifrons Edwards, 1920 of *Onchocerca* sp. and *O. gutturosa* (Grácio, 1991 and Grácio & Grácio, 2012). The vectors of *O. lupi* are still ignored. In this context, our objective was to contribute for the study of *O. lupi* and their vectors.
MATERIAL AND METHODS Wild female blackflies collected at equine species and cattle were maintaining for about 12–15 days in laboratory conditions. After, they were dissected and examined for filaria larvae. The identifications were based in Crosskey (1987) and Bain & Chabaud (1986) for blackflies and nematode larvae, respectively. Literature search was carried out in Medline and PubMed etc. for consultation of publications on *O. lupi* and their vectors.
RESULTS *O. lupi* was added to the species de *Onchocerca* known to infect aninals in Portugal.The hypothesis that blackfly species is the vector is considered.
CONCLUSIONS Our anterior studies have demonstrated that S. (W.) pseudequinum and S. (S.) nitidifrons are involved in the transmission of animal onchocerciasis in Portugal. These species are widely distributed in Algarve (Grácio, 1985) for that shall be admissible to admit that they can be involved in the transmission of *O. lupi*. In Portugal, the situation of the *O. lupi* needs to be studied in order to control the disease in dogs and the risk for human infestation.

P.1.2.009 (A)

Fluctuations in prevalence of *Taenia solium* cysticercosis in pigs in Mbeya Region, Tanzania

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Taenia solium taeniosis/cysticercosis is a serious agricultural and public health problem in Tanzania. This study aimed at describing the fluctuations in porcine cysticercosis prevalence in an endemic area. Three cross-sectional surveys were carried out in Mbeya Region, Tanzania, in November–December 2007, March–April 2012, and October–November 2012. In the first survey 300 farmers in 30 villages were visited and two of their pigs randomly sampled. In the following two surveys census sampling was used in 22 villages attempting to include all non-pregnant pigs more than 2-month old. For all surveys jugular vein blood was collected and analysed for cysticercosis antigen using Ag-ELISA. In the first survey 600 serum samples were collected. In each of the following surveys approximately 800 serum samples were collected. The first survey revealed a cysticercosis prevalence of 31% (n = 600, CI = 27–35%), in the second survey the prevalence had significantly dropped to 15% (n = 822, CI = 13–18%), and in the 6-month follow-up the prevalence had increased to 24% (n = 812, CI = 21–27%). Explanations for the observed fluctuations can only be speculated. However, in 2011 the study area suffered a decimating outbreak of African swine fever (ASF). Overlapping risk factors for ASF and porcine cysticercosis support the hypothesis that ASF may reduce cysticercosis prevalence. Also, as the Ag-ELISA assay used is not species specific, variation in transmission of other *Taenia* species could influence cysticercosis prevalence when measured by Ag-ELISA. Finally, seasonal variation in porcine cysticercosis could exist because of different seasonal production systems. The fluctuations are in contradiction with the theoretical model which predicts a stable equilibrium. Further studies are needed to determine whether the prevalence of cysticercosis has an endemic equilibrium, or in fact go through fluctuations with or without the presence of the factors described in this study.

P.1.2.010 (A)

Transcriptional profiling of a neglected parasitic protozoan: *Pentatrichomonas hominis*

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Pentatrichomonas hominis has been regarded as a commensal of the human large intestine and cecum. However, cumulated clinical evidences showed that *P. hominis* is pathogenic. In addition to human, this parasite can also infect felines and canines. The wide host range and the confirmed pathogenicity of *P. hominis* qualified this parasite a neglected tropical disease. Despite the emerging public health importance of this parasite, virtually

nothing is known about this parasite at molecular level. In the present study, we investigated the effects of temperate on the growth of *P. hominis*. Our experimental data showed that *P. hominis* can survive up to 25 days at 22°C as a pseudocyst. Moreover, these temperature-stressed cells can regain their cell division ability when return to 37°C. Next-generation sequencing analyses of the transcripts collected at 5, 15, 25 days generated a reference *P. hominis* reference transcriptome containing more than 40 000 putative transcripts. We also developed a reference proteome of the *P. hominis* trophozoite by using two-dimensional gel electrophoresis. More than 600 spots can be identified based on the reference transcriptome dataset. In conclusion, the transcriptome and the proteome datasets established in the present study will provide a foundation for the ongoing whole genome sequencing project and comparative transcriptomic/proteomic analyses to provide potential drug targets against *P. hominis* infection.

P.1.2.011 (A)

Neurocysticercosis in epileptic patients in southern Rwanda

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The central nervous system infection by *Taenia solium* larvae – neurocysticercosis (NCC) – is a preventable and treatable cause of epilepsy. The role of NCC in epilepsy differs geographically and in sub-Saharan Africa, it is poorly defined. We aimed at contributing data for Rwanda, assessing factors associated with NCC, and evaluating a real-time PCR assay in case detection. In southern Rwanda and at three healthcare facilities, 215 epilepsy patients and 51 controls were clinically examined, interviewed, and tested by immunoblot for cysticerci-specific serum antibodies. In addition, patients' cerebrospinal fluid (CSF) samples were tested for anticysticercal antibodies by ELISA and for parasite DNA by PCR. Cranial computer tomography scans were available for 12% of patients. The Del Brutto criteria were applied for NCC diagnosis. Cysticerci-specific serum antibodies were found in 22% of epilepsy patients and 4% of controls ($P = 0.003$). Seropositivity was associated with age, pork consumption, and, at statistical borderline, lack of safe drinking water. 23% of the epilepsy patients had NCC (definitive, 7.4%; probable, 15.8%). In CSF samples from NCC patients, anticysticercal antibodies were detected in 10% and parasite DNA in 16% (in definitive cases in 44%). Immunoblot-positive NCC patients were older (medians, 30 vs. 22 years), more frequently had late-onset epilepsy (43.5% vs. 8.5%), and suffered from fewer episodes of seizures (each, $P < 0.001$) than patients with other causes of epilepsy. Despite some limitations, this study shows that NCC contributes to epilepsy in southern Rwanda. In-depth investigations into porcine and human cysticercosis as well as hygiene measures and health education and for *T. solium* control are required. For NCC diagnosis, PCR might provide an additional, specific tool.

P.1.2.012 (A)

Activation of sonic hedgehog play an important role in the protection of astrocytes from the infection of *A. cantonensis*

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Angiostrongylus cantonensis is a parasite of rats. It is an important causative agent of eosinophilic meningitis and eosinophilic menigoencephalisis in humans. After infecting the central nervous system, this parasite not only induces immune responses such as eosinophilia recruitment and cytokine release but also causes ROS generation and apoptosis. Astrocytes are the most abundant glial cells in the brain and have been considered to be one of the important sources of the factors that stimulate neuronal cell differentiation. In astrocytes with injuries or under oxidative stress, Sonic hedgehog signaling (Shh) pathway is activated and highly expressed. The activation of Shh leads to reduction of astrocytes apoptosis. In the present study, young adults of *A. cantonensis* were found to induce immune responses and Shh pathway activation after migration to the brain tissues of infected mice. In astrocyte cell lines, we found that treatment with 500 μg/ml *A. cantonensis* soluble antigen or worms for 2 h induced a significant increase in the protein levels of Shh, GFAP and Bax. Treatment with Shh recombinant proteins or cyclopamine (Shh pathway specific inhibitor) demonstrated that Shh could increase astrocytes survival in *A. cantonensis* soluble antigen treatment. In addition, increases in the expressions of Shh proteins *in vivo* were confirmed by immunofluorescence. Based on these findings, activation of Shh should play an important role in the protection of astrocyte injuries from the infection of *A. cantonensis*.

P.1.2.014 (A)

Molecular identification of *Toxocara* spp. and *Toxascaris leonina* in fecal samples of dogs from Northeast Iran

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INTRODUCTION Toxocara canis and Toxocara cati are the most frequent ascarids of canids and felides. Their larval stages may cause toxocariasis in humans, a serious zoonotic disease. Humans can be infected by ingestion of embryonated Toxocara eggs through contaminated soil, water, vegetables and foods. Both species can cause ocular and visceral larva migrans and may be highly pathogenic, particularly in children.

MATERIALS AND METHODS Fecal samples from 77 domestic and stray dogs were collected in the Chenaran region, northeast Iran, during 2009–2010. For safety reasons, all samples have been frozen at −80°C for at least 3 days before analysis. Isolated eggs (zinc chloride flotation/sieving technique) were differentiated morphologically and by species specific PCRs targeting the internal transcribed spacer 2 (ITS-2) regions of the ribosomal rRNA genes (Fahrion et al., 2011).

RESULTS Based on microscopical examinations, 33 (42.8%) samples contained ascarid eggs. Of these 11 (14.3%) were positive for *Toxocara* spp., 14 (18.2%) for *Toxascaris leonina* and 8 (10.4%) for both genera. Ten samples contained highly damaged eggs, probably due to deep freezing, were excluded from further analyses. Species specific PCRs resulted in the

identification of *T. canis* eggs in 4 (5.2%), *T. leonina* in 10 (13%), *T. canis* and *T. leonina* and also *T. cati* and *T. leonina* in 5 (6.5%) and 2 (2.6%) cases, respectively. Twelve samples were negative.
CONCLUSIONS The results of the presented pilot study suggest that dogs play an important role in the environmental pollution with *T. canis* and, due to coprophagy, also with *T. cati* eggs. Therefore, the high dog population density in the Chenaran area along with the long-term survival capacity of Toxocara eggs in the environment provide suitable conditions for the transmission of these zoonotic parasites in this region.

P.1.2.015 (A)

Molecular identification of *Dientamoeba fragilis* in individual referred to Milad Hospital, Tehran, Iran

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INTRODUCTION *Dientamoeba fragilis* causes wide range of symptomatic or asymptomatic infection. The occurrence of *D. fragilis* in individual referred to Milad Hospital in Tehran was determined in the present study.
MATERIAL AND METHODS In a cross-sectional study, 200 triple fecal samples were collected from people referred to the clinical laboratory of Milad Hospital during March to July 2011. All specimens were investigated by direct smear and formalin-ether method, culture in Hsre + S medium, modified iron-hematoxylin staining and polymerase chain reaction (PCR) assay targeting the 5.8S rDNA or SSU rDNA of *Dientamoeba fragilis*.
RESULTS Dientamoeba was not found using direct smear and formalin-ether methods. Only two cases (1%) were detected through culture technique. Modified iron-hematoxylin staining were revealed 10 (5%) Dientamoeba. Amplification of SSU rDNA and 5.8S rDNA showed 12 (6%) and 27 (13.5%) Dientamoeba infections, respectively.
CONCLUSION This study demonstrated high frequency of *D. fragilis* in the investigated population. Thus clinical diagnostic laboratories should be concerning this parasite in the routine stool examination. It can be concluded that Polymerase chain reaction (PCR) is a sensitive method, especially using 5.8S rDNA gene cause to amplification of small fragment increase sensitivity for diagnosis of Dientamoeba in clinical samples.

P.1.2.016 (A)

Usefulness of HDP2 and PTsol9 repetitive DNA sequences for the neurocysticercosis/taeniasis diagnosis by PCR

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INTRODUCTION Neurocysticercosis is spreading across the whole world due to the increase of migration, tourism and globalization. It is estimated that 50 million people are infected with the taeniasis/cysticercosis complex and annually 50 000 die. Therefore, we evaluated the usefulness of the two repetitive elements (HDP2 and PTsol9) as PCR targets for the diagnosis of taeniasis/cysticercosis complex in clinical samples from patients residing in Spain.
MATERIAL AND METHODS Cerebrospinal fluid (CSF), tissue biopsies, proglottids and stool samples from patients attended in Spanish hospitals, between August 2012 and April 2013, were included ($n = 35$). DNA was extracted using QIAamp DNA/Stool Mini Kit. Analytical sensitivity and specificity of the two targets were compared for both *Taenia saginata* and *Taenia solium* DNAs. Conventional PCR for PTsol9 and conventional versus seminested PCR for HDP2 were evaluated.
RESULTS The HDP2 seminested PCR had an analytical sensitivity of 100 fg for both *T. solium* and *T. saginata* DNAs, while PTsol9 PCR allowed the detection of 10 fg and 10 ng from *T. solium* and *T. saginata* DNAs, respectively. Five cases of neurocysticercosis were confirmed by PCR, four patients from Latin America and one from Spain. In others three cases, patients from Spain, France and Paraguay, *T. saginata* was identified as the causative agent of taeniasis.
CONCLUSION The HDP2 sequence used in a seminested PCR protocol had similar sensitivity to detect DNA from *T. saginata* and *T. solium* in any kind of samples. PCR on PTsol9 repetitive element showed a higher sensitivity for *T. solium* DNA detection than for *T. saginata* DNA. The combined use of these DNA targets could be useful for the differential molecular diagnosis of the taeniasis/cysticercosis complex in samples with scarce DNA such as CSF or stool samples. The neurocysticercosis/taeniasis complex is an important problem of public health for immigrant and European citizens.

1.3 Vector borne infections in Europe

P.1.3.001 (B)

Chikungunya virus infections imported in Belgium in the post Indian Ocean Islands outbreak era

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In 2006 Belgium was confronted for the first time with imported chikungunya virus infections mainly from people who visited Reunion Island, Mauritius, Madagascar and Seychelles. In this particular year, 38 out of 160 samples examined (23.8%) showed evidence for a chikungunya virus infection. Surveillance of chikungunya virus infections was performed by examining serum samples from travelers presenting at the outpatient clinic of the Institute of Tropical Medicine (ITM) Antwerp, Belgium or serum samples that were submitted by Belgian laboratories to the Central Laboratory of Clinical Biology of ITM for the diagnosis of chikungunya. Testing was performed by serology (immunofluorescence) on all samples and by real time PCR on samples from the first 10 days of illness. Clinical information was obtained retrospectively. In the 6 years following the Indian Ocean Islands outbreak (the period 2007–2012) 1407 serum samples from returning travelers were investigated. Chikungunya virus infections were diagnosed in 45 patients (3.2%). Sixty-nine percent of the patients were female (M:F = 1:2.2). The median age of the patients was 42 years. RNA could be detected in two patients. Chikungunya virus infections were reported in patients coming from Sri Lanka as from 2007, Indonesia as from 2008, Thailand and Myanmar as from 2009, Vietnam and the Democratic Republic of Congo as from 2010 and Congo-Brazzaville as from 2011. In 2012 the Democratic Republic of Congo accounted for the majority of travel destinations of patients with a chikungunya virus infection. After the emergence in 2006 chi-

kungunya virus infections are continuously registered in import medicine at a low level (6–10 infections yearly). The geographical origin of the imported infections reflects the spread of the virus over the globe.

P.1.3.002 (B)

Angiostrongylus vasorum in Slovakia

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Angiostrongylus vasorum is a parasitic metastrongyloid nematode that may cause cardiopulmonary disease, neurological symptoms and coagulopathies in dogs and cats. The parasite has an indirect life cycle with molluscan as intermediate host where infective larval stage develops. Recently, *A. vasorum* has been repeatedly reported in dogs even outside endemic areas, indicating that this parasite evidently is widely distributed over Europe. This is the first record of autochthonous infection in a dog from Slovakia that was casually diagnosed during routine preventive parasitological examination. *Angiostrongylus vasorum* first-stage larvae were consequently recovered using Baermann technique and identified by length and characteristic tail morphology. The animal originates from Slovakia and had not travelled abroad. The dog is regularly walked on grass fields with the concentrated presence of common species of frogs and Gastropoda (slugs and snails) and according to owner like to eat grass and licks everything it finds. The first finding of *A. vasorum* infected dog in Slovakia has confirmed that the parasite is spreading beyond the traditional hyperendemic foci which should be taken into consideration for veterinarians in clinical practice. Epidemiological research continues in the area.

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P.1.3.003 (B)

Survey of canine dirofilariosis in previously infection-free region of Slovakia

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Dirofilariosis was even decade ago considered as disease typical for subtropical climate and in northern European countries only sporadic cases of imported infections were recorded. Nevertheless, during the last years a significant reversal has occurred and autochthonous infections in Eastern and Central Europe have been reported. In Slovakia, *Dirofilaria* spp. was for the first time diagnosed in dogs in 2005 and in 2007 the first systematic epidemiological research was initiated. Between 2007 and 2012 a total of 3043 dogs of various age, gender, breeds and utilization were examined. Microfilariaemia was diagnosed using modified Knott test; PCR approach was used for *Dirofilaria* species identification. Dirofilariosis was detected in 369 of examined dogs with mean prevalence rate of 12.13%. *Dirofilaria repens* was confirmed as etiological agent of infection in all positive samples, in eight dogs mixed infection with *Dirofilaria repens* and *Dirofilaria immitis* (heartworm) was revealed. Endemic areas with prevalence higher 34% were identified in south-western and south-eastern regions of Slovakia. Outdoor keeping and age higher 3 years become evident important risk factors. High infection rate (more than 50% in endemic regions) found in police dogs has represented serious veterinary problem. On the other hand, very positive fact is that intensive cooperation with police veterinarians and introduction of preventive measures led in significant decrease of *Dirofilaria* prevalence with the average of 4–6%. With regard to the zoonotic character dirofilariosis represents not only veterinarian but also public-health problem. The first human case of subcutaneous dirofilariosis in Slovakia was diagnosed in 2008 and to this day four other cases were reported.

The work was realized within the frame of the project funded by the Science Grant Agency VEGA 2/0011/12.

P.1.3.004 (B)

Integrated surveillance West Nile OF, dengue and chikungunya in Veneto Region, Italy, 2010–2012

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BRIEF INTRODUCTION Since 2010 Veneto region (North-Eastern Italy) planned a special integrated surveillance of summer fevers -to increase the detection rate of imported chikungunya and dengue cases in travellers from endemic areas and promptly identify potential autochthonous cases -to detect autochthonous cases of West Nile Fever (WNF), besides those of West Nile Neuroinvasive Disease (WNND) that were already included in surveillance.

MATERIALS AND METHODS Human surveillance: Case definitions are reported in a flow chart. Entomologic surveillance: for West Nile it was carried out from May through November placing CDC-CO2 traps in five provinces of Veneto

Table 1 Cases of the three diseases notified in Veneto Region during the surveillance period

Year (15th June–31st October)	Imported dengue cases	Imported chikungunya cases	%	Autochthonous WNF cases	%	Autochthonous WNND cases
2008	2	1		1		5
2009	4	0		0		7
2010*	14/79	1/79	18.9	4/38	10.5	3
2011	3/29	0/29	10.3	3/51	5.8	10
2012†	7/126	2/126	7.1	17/319	5.3	21

*Surveillance only started the last week of July.
†Surveillance was prolonged until 30th of November.

Region, while for dengue and chikungunya it was also performed around residences of viremic cases.
Results and Conclusions Human surveillance: Main results are summarised in Table 1. Briefly in 2012, 126 patients with fever after travelling were screened, of which 9 (7.1%) were found infected (seven with dengue and two with chikungunya). No autochtonous case was detected. Autochtonous patients screened for WNF were 319, and 17 (5.3%) resulted positive. Entomologic surveillance: WNV was found in Culex pipiens from 2010 to 2012, in four provinces. Vector index ranged from 0.25 to 0.67 and correlated with an increasing number of human cases in the following 15 days ($R = 0.843$, $P < 0.01$). No infected Aedes albopictus with dengue or chikungunya virus was found. Veneto is the only Italian region reporting WNV human cases every year since 2008. WNV is likely to cause sporadic cases and unforeseeable outbreaks for decades. Including WNF in surveillance provides a more reliable picture of WNV circulation. Timely detection of dengue and chikungunya should prompt vector control measures to prevent local outbreaks.

P.1.3.005 (B)

Short oral therapy with oleylphosphocholine is safe and effective to treat *Leishmania infantum* canine leishmaniasis

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Introduction *L. infantum* visceral leishmaniasis (VL) is prevalent in European countries of the Mediterranean basin and affects both humans and dogs. We have previously demonstrated that oleylphosphocholine (OlPC) can induce parasitological cure in the hamster model of *Leishmania infantum* VL after 10-day oral treatment. The present study analyzes the non-clinical safety of OlPC and evaluates its clinical efficacy in dogs naturally infected with *L. infantum*.
Methods Oral doses of 1.5, 4 and 8 mg/kg were administered to healthy dogs for a 28-day period to assess tolerance and toxicity. Based on these results, a dose of 4 mg/kg/day was selected to treat for 14 days a cohort of eight naturally *L. infantum* infected shelter dogs. The dogs were assessed at the clinical and parasitological levels at the beginning of treatment (day 0) and on days 15, 30 and 90.
Results In healthy dogs dosing of 1.5 mg/kg did not produce any adverse events, but some dose-dependent vomiting was noted at the two other doses, although reversible. No organ toxicity was detected at any dose tested. In the cohort of infected dogs, three dogs experienced mild gastrointestinal side effects while all other dogs tolerated the treatment without any side effect. Treatment with OlPC induced fast clinical improvement in all dogs in terms of weight gain and reduction of clinical symptoms. All dogs were considered clinically cured on day 90. PCR analysis failed to detect parasite DNA in the blood on day 15, but detailed examination indicated that the bone marrow still contained low numbers of parasites.
Conclusion This proof-of-principle study shows that short oral treatment with OlPC is safe and improves clinical signs of *L. infantum* canine leishmaniasis. Additional studies are needed to optimize dosing regimen and to assess long-term treatment efficacy.

1.4 Neglected Tropical Diseases

1.4.2 Dengue

P.1.4.2.001 (B)

Influence of climate change in the definition of sex in *Aedes aegypti* and its impact on dengue epidemics

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According to the WHO, dengue is considered as the most important viral diseases transmitted by mosquitoes. According to the report of climate change and biodiversity, it is estimated that the average temperature of the earth's surface will rise between 1.4 and 5.8°C by the end of the twenty-first century. The aim of the study was to establish the influence of the environmental average temperature in the determination of the sex of the *Aedes aegypti*, since it has been demonstrated in some species of lizards and tortoises, and its influence on dengue epidemics. So, it was performed a correlational ecological study and a experimental study. The first one calculated the regression linear model between the environmental temperature of Neiva, Colombia, from 2008 to 2010 and the incidence of dengue in the same period. The regression model calculated for the year 2009, 33.10% of the effect of dengue was explained by the environmental temperature (Prob > $F = 0.0007$). For the year 2010, the regression model explained the 26.05% of the incidence of dengue because of the environmental average temperature (Prob > $F = 0.0059$). According to the model, the increase of 1°C of the environmental temperature explained the increase in 11.78 cases of dengue. In the experimental study, 600 eggs of *Aedes aegypti* were exposed to 26, 28 and 32°C and the sex was determined until the second generation. It was calculated the Odds Ratio between the gotten results to different exposure temperatures, finding that the chance of being female versus male at 26°C was 0.78 times (CI = 0.52940–1.16612), meanwhile it was 78% (CI = 1.24238–2.55775) at 28°C. Therefore, it is considered a possible relationship between environmental temperature to 28°C and the increase in the incidence of dengue by a rise in the population of females of *Aedes aegypti*.

P.1.4.2.003 (A)

Increasing number of imported dengue fever cases in Denmark

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Introduction We report on a retrospective study that aimed to: Identify patients with imported Dengue at Rigshospitalet, Denmark from January 2009 to March 2013. Characterize the dengue patients with regard to gender, age, month of travel and country in which the infection was acquired. Evaluate the performance of SD-Bioline Dengue Duo NS1 + AbCombo (Standard Diagnostics INC, Korea) compared to RT-PCR.
Material and Methods Six hundred and fifty-four patients who had travelled in Dengue-endemic areas presenting with febrilia were identified. SD Dengue Duo were performed on all samples from August 1. 2012 and on selected frosen samples with IgM reactivity or a PCR positive result. A number of samples have been tested at a reference laboratory (SSI) with

RT-PCR and IgM/IgG. Comparison was done on samples with no more than 3 days apart.
RESULTS Our study showed an increase in cases of Dengue fever in recent years compared to previous studies. David KP et al found 44 cases between 1988 and 1998, whereas we saw 69 cases in a 4-year period, with an average of 20 cases 2010–2012. The majority of these cases were imported from Thailand by adult men. When comparing the Dengue Duo test to RT-PCR, we found a good correlation (Table 1) on 47 samples tested in both assays. The sensitivity was 100% and specificity was 97%.
CONCLUSIONS A marked increase in the number of imported Dengue cases were found in the years 2010–2012 with an average of 20 cases per year. A comparison between the NS1 and PCR showed a high sensitivity and specificity for the Dengue Duo NS1 + AbCombo.

NS1Ag/PCR	Positive	Negative	Total
Positive	17	1	18
Negative	0	29	29
Total	17	30	47

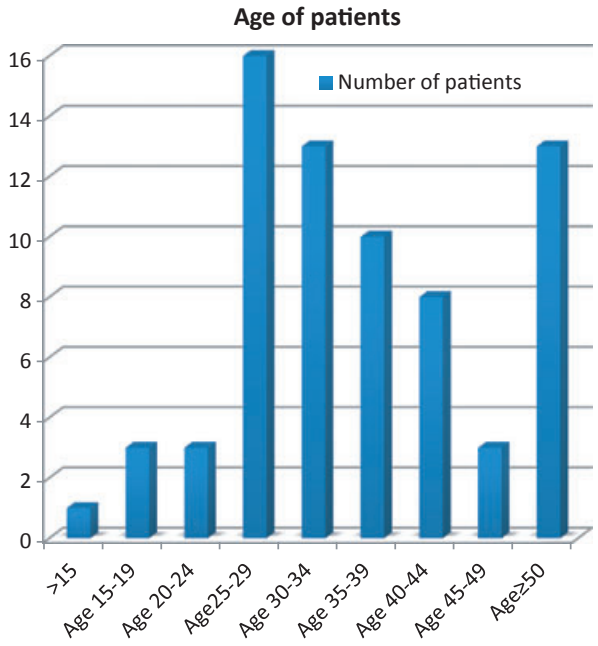

Figure 1

Figure 2

P.1.4.2.004 (B)

The dengue outbreak in Madeira 2012: exploring the origins

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INTRODUCTION The incidence of dengue has increased rapidly with increasing geographic expansion to new countries. In 2012, the first major dengue outbreak occurred in Madeira, Europe (ever since the 1927 outbreak in Greece) with over 2100 cases. We attempted to explore the origin of this outbreak by investigating the probable pathway of importation via international air passengers to Madeira. We also sourced for the dengue virus sequence to help identify the most likely country of origin.
METHODS We used the Madeira (KC248375) sequence and aligned it to existing dengue gene banks. To describe global air travel patterns to Madeira from dengue endemic countries, we analyzed worldwide full-route flight itinerary data from the International Air Transport Association (IATA) between January 1st 2005 and December 31st 2010.
RESULTS Sequencing indicated that the Madeira virus originated from South America and is most closely related to a DENV-1 strain from Colombia/Venezuela (GQ868570). There were 24 722 air passengers from dengue endemic countries to Madeira in 2010. While 18 documented dengue endemic countries were the source of all international travelers to Madeira, just two (i.e. Venezuela and Brazil) were the source of 97.6% of all arriving international travelers (Venezuela to Madeira: 19 767 air passengers, Brazil to Madeira: 4351). Only Venezuela has direct flights to Madeira. Taking number of air passengers into account and the dengue endemicity in the country of origin, 99 passengers from Venezuela may have imported dengue into Madeira in one year compared to 21 passengers from Brazil.
CONCLUSION Based on interconnectivity via air travel between Madeira and dengue endemic countries, Venezuela is the most likely country of origin for the importation of dengue into Madeira. This was confirmed by dengue sequencing. Using international air travel patterns may aid in identifying the most likely origin of importation into currently non-dengue countries.

P.1.4.2.005 (B)

Larvicidal activity of Mentha × villosa Hudson essential oil and derivatives

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INTRODUCTION It has been reported that essential oils and derivatives present toxic activity against mosquito larvae *Aedes aegypti* L. Therefore, these facts led us to investigate the larvicidal potential of Mentha × villosa Hudson essential oil (MVEO), its major constituent, rotundifolone, and 15 analogues compounds against larvae of *A. aegypti* to identify the molecular characteristics which contribute to the larvicidal effect of these derivatives.
METHODS AND MATERIALS Third-instar larvae were used in the experiment. The concentration ranges were determined by a previous curve concentration-response with 20 larvae. A 20 000 ppm stock solution was prepared using each compound (20 mg/ml), Tween-80 (10% v/v), DMSO (30% v/v), and natural mineral water (60% v/v). The stock solution was used to make 20 ml water solutions ranging from 10 to 500 ppm. Adequate volumes of the stock solution were transferred to empty disposable cups, followed by the addition of previously separated larvae. A mortality count was conducted 24 h after treatment. Controls were prepared with Tween-80 (0.1 ml), DMSO (0.3 ml), and water (19.6 ml). Three replicates were used for each concentration and the control.
RESULTS The MVEO exhibited outstanding toxic effects against *A. aegypti* larvae (LC50 of 45.0 ppm). All tested compounds were less potent than rotundifolone.
CONCLUSIONS Functional groups and their positions in the p-menthane skeleton influence the larvicidal activity, suggesting that appropriate structural modifications may be possible to develop new larvicide agents.

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P.1.4.2.007 (B)

Spatial distribution of age change among dengue patients in Thailand

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INTRODUCTION Dengue remains a public health problem in Thailand and other countries in tropical and temperate zones. In recent years, reports have shown an increase in the age of dengue patients at the country level in Thailand. However, no formal study had been conducted on the change of age among dengue patients at the local scale over time. Age distribution among dengue patients is important when planning and implementing disease prevention and control measures. Our study was conducted to determine the age change spatially among dengue patients at the district level in Thailand.
MATERIALS AND METHODS Plaeng Yao District, Chachoengsao Province, in eastern Thailand was selected as the study site. The age of dengue patients admitted to Plaeng Yao Hospital was divided into two groups: (i) ≤15 years, and (ii) more than 15 years. Global Positioning System (GPS) units were used to record the locations of dengue patients between 1999–2002 and 2009–2012 in Plaeng Yao District. Spatial maps of the age of dengue patients were created using Kernel estimation.
RESULTS AND CONCLUSIONS Spatial maps created by using the Geographic Information System (GIS) showed that both age groups of dengue patients were highly concentrated in the Northeast area of Plaeng Yao District. The average age of dengue patients between 1999–2002 and 2009–2012 were 13.00 ± 7.51 years (range = 1–40) and 22.45 ± 13.09 years (range = 1–74) respectively. The age of dengue patients increased significantly over time. This study was the first to use GIS spatial analysis to compare the age of dengue patients over time. Results obtained could be used for planning effective dengue prevention and control programs.

P.1.4.2.008 (A)

Epidemiology of dengue haemorrhagic fever in Sri Lanka

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Dengue was first serologically confirmed in Sri Lanka in 1962, with the first outbreak being reported in 1965. Thereafter, multiple similar outbreaks with endemic dengue fever (DF) were reported in urban areas, with only occasional reporting of Dengue Haemorrhagic Fever (DHF) a potentially life-threatening condition. DHF first emerged as a public health problem in Sri Lanka from 1989. Since early 2000 progressively larger epidemics of Dengue with more DHF have occurred at regular intervals. Here, we report major epidemiological shift with increasing incidence of DHF being reported in the country. Dengue has been a nationally notifiable disease since 1996. Characteristics of reported DHF patients and deaths from 2009 to 2012 are presented from national surveillance data. Dengue reporting has intensified during the past decade with expanding geographic distribution, increasing epidemic frequency and the emergence of DHF in new areas. A major upward shift with a mean incidence of 177.5 cases per 100 000 population was reported (both DF and DHF) over the past 4 years. During the same period clinically confirmed cases by severity showed a markedly high proportion of 10–15% DHF at national level. Although DHF was reported among all age groups they tend to be younger with a mean age of 14 years compared to 18 years among DF patients. Overall case fatality rate (CFR) has declined from 1% in 2009 to 0.4% in 2012. Mean age of deceased DHF patients was higher than those who survived. There is an increase incidence of DF and DHF observed in Sri Lanka. Overall case fatality due to DHF has shown a gradual decline. Therefore, we conclude that the national guidelines on management and the serial blood counts beyond day 3 of fever introduced in 2009 has helped early identification of DHF and proactive management leading to a reduction in mortality.

Abstracts of the 8th ECTMIH and 5th CSBSP

P.1.4.2.009 (B)

Dengue epidemiology in a high-income urban setting: outbreak investigations from Kaohsiung City, Taiwan

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Kaohsiung City, a modern metropolis of 1.5 million people, has been the focus of dengue activity in Taiwan for several decades. Our study aimed to provide a temporal and spatial description of dengue epidemiology in Kaohsiung City, based on data from the routine surveillance system conducted by Taiwan CDC. The system ensures reporting and laboratory confirmation of all suspected dengue cases from passive and active surveillance activities, for the years 2003¡V2009. Age and sex dependent incidence rates were investigated along with the spatial patterns of all confirmed cases. Incidence was calculated by using the yearend population data for each study year as the denominator. The numbers of clinical manifestations were compared among the ages and between cases detected in the passive and the active surveillance system. Spatial patterns of dengue was measured by Moran¡¦s *I* and Anselin¡¦s LISA. There were 2087 laboratory-confirmed dengue cases in this 7-year survey. More than 95% of the cases were recorded during July¡VDecember of each year. We found dengue incidence rate was the lowest among children <5 years of age, increased by age, peaking in adult 55¡V64 years old. Women had slightly but not significantly higher incidence than men. Fatalities only occurred among adults older than 64 years old. Almost 75% of all confirmed cases were obtained through passive surveillance activities, while actively detected cases included immediate family members, neighbors and colleagues of confirmed cases. Cases showed a significant clustered pattern within the city, and the location of incidence hotspots changed each epidemic year. Our findings suggest the strategies of dengue prevention in Kaohsiung City should focus on elderly populations. Changing locations of hotspots implies unmeasured environmental and demographic factors needed to be explored.

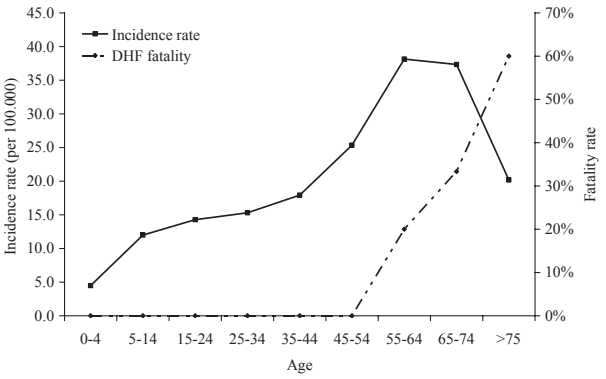

Figure 1

Table 1 The annual number and rate of dengue cases with identified serotype in Kaohsiung City, 2003–2009

	Confirmed cases	DENV-1	DENV-2	DENV-3	DENV-4	Annual no. serotype (%)
2003	62	0	1	0	0	1 (1.6)
2004	45	16	2	0	2	20 (44.4)
2005	99	2	5	37	0	44 (44.4)
2006	766	2	35	265	1	303 (39.6)
2007	153	55	2	0	0	57 (37.3)
2008	326	134	7	0	1	142 (43.6)
2009	636	1	4	280	0	285 (44.8)
Total	2087	210	56	582	4	852 (40.1)

Table 2 Clinical manifestations as reported by male ($N = 1030$) and female ($N = 1057$) dengue patients in Kaohsiung City, Taiwan for the period 2003–2009

Manifestations	Male (%)	Female (%)	Total (%)	*P*-value[+]
Fever	939 (91.2)	947 (89.6)	1886 (90.4)	0.224
Anorexia	539 (52.3)	587 (55.5)	1126 (54.0)	0.142
Headache	504 (48.9)	544 (51.5)	1048 (50.2)	0.247
Rash	505 (49.0)	455 (43.0)	960 (46.0)	0.006*
Arthralgia	458 (44.5)	466 (44.1)	924 (44.3)	0.862
Myalgia	468 (45.4)	454 (43.0)	922 (44.2)	0.253
Thirst	427 (41.5)	429 (40.6)	856 (41.0)	0.686
Diarrhea	320 (31.1)	282 (26.7)	602 (28.8)	0.027*
Nausea	211 (20.5)	328 (31.0)	539 (25.8)	<0.001*
Pruritus	209 (20.3)	230 (21.8)	439 (21.0)	0.411
Vomiting	137 (13.3)	211 (20.0)	348 (16.7)	<0.001*
Retro-orbital pain	115 (11.2)	109 (10.3)	224 (10.7)	0.529
Hemorrhagic manifestations	59 (5.7)	46 (4.4)	105 (5.0)	0.150

[+] *P*-value: difference in proportion between males and females for given manifestations.
*Level of significance $P < 0.05$.

P.1.4.2.010 (B)

Innovative dengue prevention and control using permethrin-impregnated school uniforms: experiences from a 1-year field trial in Thailand

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Introduction Many studies demonstrated that insecticide treated materials could be used for vector-borne disease control. However, an application of impregnated school uniforms in dengue control have not yet been evaluated. This study designed to test the efficacy of permethrin-impreganted school uniforms after being used and to determine the factors related to the reduction in efficacy at the household level.
Materials and Methods Twenty used school uniforms per school were randomly collected once a month from 10 schools to test the efficacy of permethrin impregnation. Questionaires related to uniform treatment habits were distributed in classes in all study schools. In addition, two students each from six schools were randomly selected for in-depth interview and weekly testing of their school uniforms.
Results and Conclusions The knock down effect and mortality test of used uniforms from treatment schools showed a dramatically decrease in efficacy within 6 weeks when compared to those of the control schools. School shirts and school skirts/pants were different in waning efficacy to permethrin after being used. Results may be related to the cloth materials of different kinds of school uniforms. Our interview results showed that 73.4% of children wore impregnated school uniforms 5 days a week. The majority of them washed the uniforms once a week (68.7%) mainly with washing machines (62.4%) and power detergents (90.2%). Approximately 41.1% dried the uniforms under shadow or windy area and 78.4% ironed the uniforms. The majority of school children had no activity after school (62.5%), therefore they changed from school uniforms to casual clothes after school (63%). Our interview results revealed that uniform treatment habits may be important factors for the reduction in efficacy of permethrin-treated uniforms over time. Improvement of impregnation is needed to prolong the impact of treated uniforms on mosquito vectors and dengue transmission.

P.1.4.2.011 (B)

Deciphering the origin of dengue virus introduced in Madeira, Portugal

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Dengue is caused by four different but antigenically related viruses, DENV-1 to DENV-4, transmitted to humans through the bites of Aedes mosquitoes. The disease is endemic in 100 countries from Asia, America, Africa and Oceania. In Europe, in the past century, Dengue epidemics occurred in Greece and other Mediterranean countries where *A. aegypti* was present. After that, it disappeared from Europe, but another competent vector, *A. albopictus*, was introduced in late 70s. In recent years, *A. aegypti* was reintroduced in south Russia and in Madeira island (Portugal). In 2010, dengue re-emerged in the French Riviera and Croatia, with small outbreaks. Two years later, in October 2012, a sustained and explosive epidemic appeared in Madeira Island. More than 100 travellers from different European countries acquired the infection after returned from the island. Preliminary analyses revealed that the outbreak was caused by DENV-1 possible introduced from Latin America. The aim of this study was to determine the most probable origin of the dengue virus introduced in Madeira, Portugal in 2012. We analyzed the E/NS1 junction and complete envelope sequences from imported dengue infections acquired by returning travellers from Madeira and Latin American countries. Phylogenetic analysis revealed that all DENV-1 strains belong to genotype V. The strains introduced in Madeira clustered within one of the South American sublineages, represented by strains isolated in Colombia and Venezuela in recent years but close related to a strain detected in a spanish traveller returning from Venezuela in 2012. In conclusion, our data suggest that at phylogenetic level, the most probable source of the DENV-1 introduced in Madeira island is Venezuela.

P.1.4.2.012 (B)

Dengue infection in domestic dogs in urban Thailand and its implications for dengue surveillance and control

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Introduction Dengue is an arboviral disease transmitted by Aedes vectors. Many studies have focused on the dengue virus in humans and mosquitoes, but only a few have targeted primates or other domestic animals that likely could be reservoirs of dengue virus. Since domestic dogs share both habitats and vectors with humans, our study used a one health approach to investigate the relationship between dengue and canines, which could improve dengue surveillance and enhance the efficiency of dengue prevention and control.
Materials and Methods Human relationships with domestic dogs were investigated by use of a questionnaire interview in the urban city of Nakhon Sawan, Thailand. Analysis was performed using PASW Statistic 18.0. Dog sera were collected and screened for dengue infection using RT-PCR. Positive samples were then inoculated in cell lines for virus isolation. Partial nucleotide sequences obtained from positive supernatants were aligned and a phylogenetic tree was constructed by the Neighbor-Joining method.
Results and Conclusions A total number of 1041 households with dogs were interviewed, and 632 dog sera were collected and screened for dengue viruses. Six samples (0.95%) were positive and four out of six were isolated. They were identified as DENV-2 and DENV-3. Phylogeny analysis revealed that these isolated samples were closely related to those strains circulating in human populations. Our interview results showed that the dengue-positive dogs were poodle breed (50%), which were raised indoors (50%), and Thai (33%) and Thai-mixed breed (17%), which were raised outdoors (50%). Health education was implemented in households with dengue-positive dogs to raise awareness among householders to avoid

interactions with dogs in the presence of Aedes vectors during the day-time. Our findings could be applied to enhance dengue prevention and control by establishing a new surveillance system to focus not only on vectors but also potential reservoirs, such as domestic dogs.

P.1.4.2.013 (B)

Cost-effectiveness of a novel technology for dengue prevention in children: insecticide-impregnated school uniforms

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Children carry a larger proportion of the disease burden of dengue in endemic countries and are more likely to experience the complications of the disease, including dengue haemorrhagic fever (DHF), compared to adults. Prevention is key to reducing morbidity and mortality because no specific curative treatment exists for the disease. Vector control using adulticides and larvacides has shown some but very limited impact on dengue incidence. The implementation of vector control activities is particularly challenging in urban and peri-urban areas, where the vector is well-established and thriving on proximity to humans. The search for novel, acceptable and affordable methods of vector control is ongoing. Because dengue vectors bite primarily during the day, schools – where children spend most of their day – are an ideal setting for dengue prevention and control. A community-based controlled trial is currently underway in eastern Thailand to assess the impact of impregnated school uniforms on the incidence of dengue in school-aged children. While awaiting the results of the trial, a recent modeling study has shown that 'the use of insecticide-impregnated uniforms has an efficacy varying from around 6% in the most pessimistic scenario to 55% in the most optimistic scenarios simulated'. Following standard guidelines of economic analyses, we will develop a decision analytical model to evaluate the potential health and economic value of this new intervention from the societal perspective, using data from the published literature specific to Thailand. The model will simulate the incidence of dengue fever and DHF, survival, disability adjusted life years, and costs in a hypothetical cohort of children receiving the intervention and will facilitate a comparison against scenario of no intervention. The outcome of this analysis will be expressed as a ratio of incremental costs to incremental health outcomes of the intervention. We will perform sensitivity analysis of key variables and assumptions.

P.1.4.2.014 (B)

Development of a simple single-tube reverse transcription-loop-mediated isothermal amplification assay for detection of dengue virus from clinical samples

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Brief Introduction Early and rapid detection of dengue virus (DENV) infection in patients during febrile phase can greatly improves the patient management and prevent disease spread. An easy to perform, low cost and highly sensitive method is desired to enable the routine practice of dengue diagnosis in the rural areas where resource is limited.

Material and Methods A single-tube reverse transcription-loop-mediated isothermal amplification (RT-LAMP) assay was developed for the detection of all four DENV serotypes. The sensitivity and specificity of the RT-LAMP were assessed. The performance of RT-LAMP for DENV RNA detection in clinical samples was also evaluated and compared against the quantitative reverse transcription-polymerase chain reaction (qRT-PCR), IgM- and IgG-capture enzyme-linked immunosorbent assays (ELISA).

Results Out of 305 clinical samples, acute dengue infection was confirmed in 172 samples; 44% and 47% of which were positive for DENV using RT-LAMP and qRT-PCR, respectively. The use of either RT-LAMP or qRT-PCR in combination with the dengue IgM and IgG ELISA improved detection of acute DENV infection to over 90% compared to only 70% when dengue ELISA were used alone. The RT-LAMP assays showed high concordance with the qRT-PCR. The RT-LAMP assay detected as low as 10 copies of viral RNA within an hour but 100% reproducibility was achieved with 100 copies. There was no cross reactivity of RT-LAMP with Japanese encephalitis virus, Chikungunya virus and Sinbis virus.

Conclusion The RT-LAMP assay developed in this study is simple, sensitive and specific for detection of DENV RNA in clinical samples. Without the need of costly equipment, the assay can be easily performed for routine dengue diagnosis in resource-limited settings where dengue is endemic.

P.1.4.2.015 (B)

Residual effect of pyrethroids used in vector control on different house materials in the Peruvian Amazon

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The use of insecticides is crucial in the control of vector-borne diseases. In the case of malaria two are the most common strategies used in the Peruvian Amazon: The Indoor Residual Spraying (IRS) and the use of insecticide-impregnated mosquito nets. for dengue control, Rapid spraying of insecticides inside the houses is a common practice. In the Peruvian Amazon, houses are constructed with different type of materials, depending on the availability and economical situation of the inhabitants. Although houses made of bricks are found in urban areas, wood is the most commonly used material in peri-urban settlements. The objective of this study was to determine the residual effect of pyrethroid insecticides in house walls constructed with bricks, painted wood and unpainted wood. Cone bioassays were carried out monthly from March to July 2012, using an *Aedes aegypti* colony. The first three months higher mortality was observed in brick walls (96.7%, 91.7% and 83.3%) compared to wood-made houses. However, four months after the last application mortality was close to 50% in all materials used. The residual effect of pyrethroids last longer in unpainted compared to painted wood.

P.1.4.2.016 (B)

Dengue fever acquired during occupational stay in the Maldives

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INTRODUCTION There have been diagnosed 107 cases of dengue fever (DF) at the Department of Infectious Diseases of Hospital Na Bulovce in Prague since 1/2004. The vast majority of them have been diagnosed in tourists; however, the proportion of occupationally exposed patients with DF has significantly increased since 9/2012.
METHODS Retrospective study evaluated clinical and laboratory characteristics of DF in Czech patients who had worked on construction site on Fushivelavaru (the Maldives). All cases were identified through data review in co-operating diagnostic laboratories. A case of dengue infection was diagnosed by the serology and/or presence of NS1 antigen/RT-PCR.
RESULTS The infection with dengue virus imported from the Maldives was confirmed in 33 persons (29 M and 4 F) with age median 38 years (IQR 35–46) since 9/2012. Only three patients (9.1%) required hospital stay. The NS1 antigen has been detected in 19 patients (57.6%) and RT-PCR was positive in 11 (33.3%). The median period from symptoms onset was 5 days (IQR 4–7) in NS1+ cases and 4 days (IQR 3–5) in RT-PCR+, $P = 0.171$. In the NS1+ patients there have been described: leukocytopenia (median 3.9×10^9/l; IQR 3.3–4.6), thrombocytopenia (134×10^9/l; IQR 96–251) and low CRP (3.3 mg/l; IQR 1.9–9). The most frequently observed symptoms included fever (all patients), muscle and joint pain (91.6%), headache (69.2%) and rash (64.3%). The clinical course was uncomplicated in all patients.
CONCLUSION The vast majority of DF cases after stay in tropics is diagnosed among tourists, however, presented study underlines the risk, which dengue represents to occupationally exposed persons. The infection in the serologically naïve hosts is usually uncomplicated, however, infected persons are at significant risk of development of severe dengue if challenged to infection with other serotype. Furthermore working incapability represents significant financial burden to the employers.

P.1.4.2.017 (B)

Flight travel related risk of dengue introduction in Mediterranean cities

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BRIEF INTRODUCTION Dengue transmission has expanded geographically over the past two decades, making dengue the most widespread arthropod-borne viruses (Domingo, Niedrig et al. 2011). Increasing evidence shows European distribution of secondary dengue vector Aedes albopictus. Travelers from endemic areas risk can acquire and import dengue into European destinations. In 2012, theoretical risk transcended into the first dengue outbreak in Europe since 1928, following the 2005 establishment of primary vector *Aedes aegypti* in Madeira, Portugal.
MATERIAL AND METHODS Synthesizing annual incidence rate within endemic departure countries and estimated air passenger volume, we generated and compared risk of introduction indices across three European destination cities with established Aedes albopictus populations. We modeled risk of importation for Barcelona, Rome, and Athens using methods and data from www.vbd-air.com, IATA, and WHO regional websites.
RESULTS Rome, receiving year round direct flight traffic from 20 dengue endemic areas worldwide, has the highest risk of importation. Athens only receives direct flight traffic from two endemic Asian countries, therefore, has drastically lower risk. Barcelona has six direct flights from departure cities in dengue endemic areas of the Americas, Africa, and Asia, therefore, has considerably lower risk of importation than Rome, but higher than Athens.
CONCLUSIONS We explored potential dengue introduction into selected European destinations by integrating available knowledge and predictions regarding vector suitability, disease transmission patterns and global air passenger travel. Our findings suggest Rome has a manifold heightened risk for introduction of dengue virus to the established populations of Aedes albopictus compared with Barcelona and Athens, due to its higher passenger volume, multitude of routes to the Americas, Asian, and Africa, and environment conducive to vector survival.

P.1.4.2.018 (B)

Innovative dengue prevention and control using permethrin-impregnated school uniforms: purchasing decision based on background knowledge and experience

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INTRODUCTION Dengue is one of the important public health problems worldwide. The disease can only be prevented through vector control. Permethrin-impregnated school uniform is proposed as an alternative tool for dengue prevention and control in school-age children. In order to assess purchasing decision of impregnated uniforms, a social science study was conducted among parents from participating schools in Chachoengsao Province, Thailand.
MATERIALS AND METHODS A structural questionnaire was administered through all classroom teachers of 10 schools. Data related to determining factors to purchase impregnated uniforms were entered and analyzed in SPSS for windows version 11.5.
RESULTS AND CONCLUSION Out of 1200 interviewed questionaires, a majority (69.2%) of respondents was female and aged between 20 and 40 years (59.9%). 14.2% had experienced dengue in their families. Our results showed satisfaction of parents to impregnated uniforms. 72% mentioned that they could not smell the difference between normal and impregnated ones. Most parents (90.8%) mentioned that their children were not allergic to the impreganated uniforms. 69.1% planned to

buy impregnated uniforms in the future. The Chi square test of association showed significant correlation between purchasing decision of parents and the following factors: highest education level ($P < 0.001$), income higher than 10 000 Baht ($P < 0.05$), occupation ($P < 0.05$), dengue experience in a family ($P < 0.05$), dengue incidence in the community ($P < 0.01$), allergy to permethrin ($P < 0.01$) and odor of impregnated uniform ($P < 0.01$). Parents were more agreed to buy the impregnated uniforms if their children were not allergic (94.7% vs. 5.3%) or smelt the difference between normal and impregnated ones (77.3% vs. 22.7%). Parents who never had dengue experience decided not to buy the impregnated school uniforms more than those who had dengue experience (86.9% vs. 17.6%). From our study, if the impregnated school uniforms proved effective to prevent dengue, more parents were willing to make decision to buy them.

1.4.3 Mass drug administration and the control of neglected tropical diseases: learning from success, learning from failure

P.1.4.3.001 (A)

Scenarios for lymphatic filariasis eradication and the associated impact on demand for MDA

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Introduction Lymphatic filariasis (LF), a neglected tropical disease causing chronic morbidity, affects an estimated 120 million people in 73 countries. In 2000 the Global Programme to Eliminate Lymphatic Filariasis (GPELF) was established with the goal of eliminating LF as a public health problem within 20 years. While the program has made great gains towards controlling LF, elimination by 2020 is unlikely to be achieved.
Methods This analysis will rely heavily on the most current projections of the at-risk population for LF, as listed in the WHO's Preventive Chemotherapy databank for lymphatic filariasis, as well as from the results of deterministic modeling to predict the number of MDA rounds required to achieve local elimination in given transmission settings. The model will take into consideration different transmission archetypes, including the three different LF species (*W. bancrofti*, *B. malayi* and *B. timori*), the primary vectors (Anopheles, Culex, Aedes, Mansoni), and the different MDA protocols (albendazole with DEC or ivermectin). Varying levels of scale up and MDA coverage will be considered, with optimistic and pessimistic scenarios developed.
Results Scenarios that are more likely to achieve eradication of lymphatic filariasis than the current GPELF strategy will be developed that take into account the adaptations necessary to accommodate the disparity between different LF endemic environments. For each scenario, the total number of treatments for MDA, either administered as albendazole and ivermectin or albendazole and diethylcarbamazine (DEC), will be estimated. The number of treatments required to maintain control or to achieve eradication will then be compared against each other, as well as against the most recent GPELF's projections and planning documents in order to determine whether a higher level of commitment and resources than currently allocated will be necessary to reach eradication.

1.4.5 Genital schistosomiasis

P.1.4.5.001 (B)

A simple school questionnaire for rapid screening of urinary schistosomiasis in Yemen

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Introduction Schistosomiasis ranks second to malaria in terms of socio-economic and public health importance in Yemen. The present work was designed to assess the validity of the morbidity questionnaire as a rapidly screening tool of schoolchildren for urinary schistosomiasis as compared to the presence of eggs in urine as a gold-standard parasitological diagnosis.
Material and Methods The study sample included 696 children (305 males and 391 females) with mean age 12.5 ± 1.05 years attending primary-preparatory mixed school in Battis village, Abyan governorate, Yemen. The questionnaire is simply asked the children key questions as 'Did you have pain while urinating?'; 'Did you have blood in urine?' in the last 2 weeks and asked about the past history of previous infection with urinary schistosomiasis and history of anti-schistosomal treatment 1 month prior to the implementation of the study. The children were interviewed and their urine examined. Macroscopic examination for visible haematuria and detection of micro-haematuria by chemical reagent strips. Microscopic examination of urine samples for detection and counting eggs of *S. haematobium* per 10 ml urine.
Results One hundred and twenty six children (18.1%) were infected with urinary schistosomiasis. The diagnostic performance of self reported dysuria, self reported past history of previous infection and self reported history of anti-schistosomal treatment using the morbidity questionnaire recorded a poor diagnostic performance. The self reported haematuria, microhamaturia, and concomitant haematuria revealed a good diagnostic performance.
Conclusions The reagent strip method for detection of microhaematuria is practically cheap, valid, easy to perform and rapid assessment method for identifying infected individuals and communities at risk.

P.1.4.5.002 (B)

The genital Schistosomiasis and HIV research project (GENSHIV): effect of praziquantel treatment on genital HIV-1 RNA shedding in *Schistosoma haematobium* and HIV co-infected subjects – a randomized trial

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Introduction Worldwide 150 million people are infected with *Schistosoma haematobium*, 85% living in sub-Saharan Africa. The prevalence of HIV-1 in Africa correlates to the geographical distribution of freshwater lakes, suggesting an association to schistosomiasis. Schistosomal egg-induced lesions in the genital mucosa, seminal vesicles and prostate may facilitate entry of virus, increase blood vessel proliferation and density of HIV target-cells in the mucosa causing increased viral shedding into semen and vaginal secretions. Semen from men with schistosomiasis (MGS) contains an increased number of leukocytes which declines with Praziquantel-(PZQ)-treatment. To date no data regarding the impact of schistosomiasis-infection on

genital HIV-shedding in coinfected men is published, but the GENSHIV-study hypothesis is, that dually infected individuals pose a yet ignored risk of HIV-transmission to sexual partners and offspring, and, that PZQ-treatment will cause resolution of schistosoma-egg-induced inflammation reducing HIV-shedding.
MATERIAL AND METHODS An epidemiological questionnaire-study elaborating on schistosomias and HIV-transmission to sexual partners and children, and a clinical study treating 240 co-infected individuals with PZQ observing effects on changes in HIV-shedding will be conducted in Burma Valley, Zimbabwe where HIV and Schistosomiasis are hyper-endemic. Participants are randomized to receive PZQ at baseline or after 12 weeks. Blood, urine, vaginal-lavage, breastmilk and semen are collected prior to PZQ-treatment and at 6 and 12 weeks follow-up. Blood is analyzed for schistosoma-antigens/antibodies, HIV-RNA and CD4-count. Urine is tested for STIs and schistosoma. Vaginal lavages, breastmilk and semen samples are analyzed for schistosoma, soluble-egg-antigens, eosinophil cationic protein, HIV-1-RNA. All compartments are analyzed for presence of HIV-infected cells by p24 flow-cytometry, and comprehensive flow cytometric characterization of HIV target cells in the genital compartment is performed.

P.1.4.5.003 (B)

Improved water access and clean sanitation alone may not be enough to significantly reduce the problem of schistosomiasis and soil transmitted helminths in Ugu district, South Africa

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BACKGROUND Improving global access to clean drinking water and safe sanitation is one of the least expensive and most effective means to improve public health and save lives. In South Africa, water accessibility and adequate healthy sanitation infrastruture are regarded as basic infrastructure and human rights issue.
AIM To determine if improved sanitation and water access has led to significant reduction in prevalences of helminths in young school age going female pupils.
METHODS A cross–sectional descriptive study was conducted in Ugu district, KwaZulu-Natal amongst female pupils aged 10–12 (N = 1057) from 18 randomly selected rural schools. Demographic information, three urines on consecutive days and one stool sample were obtained from each study participant. Urine centrifugation and Kato-Katz (stool) methods were used for analysis. Information on water and sanitation in the study area were obtained in two ways: through municipality's data base (documents, files, accounting sheets of various projects etc) and through structured interviews with 15 ward councillors from communities surrounding sampled schools.
RESULTS Prevalences of *S. haematobium*, *A. lumbricoides* and *T. trichiura* were f 28.1% (95% CI:25.0–31.0%), 24.5% (95% CI:22.0–27.0%) and 25.9% (95% CI:23.0–29.0%). respectively. The majority of pupils had low to average infection with very few reporting high infection. Councillors reported that more than 80% of households in all communities have access to basic water within 200 m whilst approximately 70.0% households have proper sanitation.
CONCLUSION Findings indicate that one in four pupils may be infected with one of three targeted helminths even though the majority of the population has access to water and adequate sanitation. Living conditions of a high number of pupils still appear to lack basic infrastructure. Other interventions eg. Mass (treatment) may need to be implemented to significantly reduce infection prevalences of targeted helminths.

P.1.4.5.004 (B)

Preliminary results from an intervention study in children aged 2–16 years in controlling Schistosomiasis in a village from Bengo Province (Angola)

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INTRODUCTION Schistosomiasis, Soil Transmitted Helminths and malaria coexist as endemics and potential factors causing anemia and other clinical consequences in children and compromising not only their growth and development but also their academic performance levels. The prevention and control of this disease involve the preventive administration of Praziquantel, and Albendazol, improvements in fresh water supplies, healthcare and education. This work presents the first results of a broader project aiming to contrast the results of the effectiveness of a school based programs and those of a community based programs as well as between pre-school age children and school age children, in Bengo province, Angola.
METHODOLOGY An intervention study in a village from Angola, of 95 preschool children (2–5 year olds), 115 school-aged children (6–15 year olds) and 185 adults (>15 year olds) was conducted to evaluate malnutrition, anemia, malaria, schistosomiasis and geohelminths and treat all intervenient with Praziquantel and Albendazol.
RESULTS Malnutrition was common among children (in preschool children 13.4% were under-weight, and 55.2% stunting, and in school children 16.6% were under weight and 22.7 stunting), and anemia (<11.0 mg/dl) was found in 65% of children. Urinary schistosomiases prevalence reached 67% of preschool children and 82% of school-aged children. Geohelminth infections were common, affecting 32% of preschool children and 58% of school-aged children.
CONCLUSIONS Here we report the first results of the intervention study and the results obtained justify the implementation of the interventions for the control of these diseases and morbidities. During the present year the effectiveness of the intervention and a comparison with a school intervention in a different village will be conducted.

P.1.4.5.005 (B)

Chromatographic analysis of lipids in the haemolymph of aestivating and starved *Bulinus globosus* and *Bulinus rohlfsi* snails

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The study of the intermediate snail hosts of medical importance through development of reliable separation techniques is known to be a major breakthrough in the control of Schistosomiasis. This study therefore investigated the thin layer chromatographic (TLC) patterns of the lipids in the haemolymph of two snail species namely Bulinus globosus (Morelet) and Bulinus rohlfsi (Clessin). Aestivation, starvation, and control experiments were set up for 30 days in the laboratory by placing three groups of snails collected in dry and rainy seasons from Oyan dam, Abeokuta in

standard aestivation slope (30 *B. globosus* and 19 *B. rohlfsi*), aquarium (30 *B. globosus* and 23 *B. rohlfsi*) while control slope had 20 *B. globosus* and 15 *B. rohlfsi*. Aestivation and control slopes contained water and mixture of sand and clay (3:1) while aquarium contained water only for starvation. All the snails were fed on lettuce ad libitum for 28 days during which water was completely drained out in the aestivation slope. The aestivation slope and aquarium were left for another 30 days without lettuce. Snails were thereafter sacrificed and lipid contents in tissues and haemolymph determined. Thin Layer Chromatography (TLC) for detection of free fatty acids and esterified lipids was conducted. TLC detected cholesterol and triglyceride, Palmitic acid and Oleic acid during aestivation while stearic acid was detected during starvation. We conclude that these lipids and fatty acids may play key roles in the survival of the snails during aestivation and this might be related to not only metabolism but antigenic properties that have to do with histocompartibility of these snails with vector schistosomes.

KEYWORDS Aestivation, starvation, thin layer chromatography, *B. globosus*, *B. rohlfsi*

P.1.4.5.006 (B)

Red urine and dysuria as indicators of *S. haematobium* in high risk populations of school going age from coastal Ugu district, KwaZulu-Natal, South Africa

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BACKGROUND In South Africa, it is estimated that 2.5 million people are infected with schistosomiasis and school age children are usually the most affected group.

AIM To determine the prevalence and intensity of schistosomiasis (bilharzia) and its association with reported 'red urine' and water contact and knowledge of bilharzia from participants.

METHODS A cross-sectional description study was conducted in the south coast of Ugu district amongst female pupils aged 10–12 (N = 1057) from 18 rural schools and areas where altitude was below 300 m. Schools were selected randomly from across the region and consent was obtained from parents. Demographic information,and three urines (collected on consecutive days between 10am and 14:00) were obtained from each study participant. Urine centrifugation method was used. Girls' knowledge about schistosomiasis (isichenene in isiZulu), extent of water contact and experience of red urine were investigated.

RESULTS Prevalence of *S. haematobium* was found to be 28.1% (95% CI:25.0–31.0%) and mean intensity was 16.7 eggs/10 ml urine. When asked whether they knew about schistosomiasis, whether they had had red urine in the past week and if they had ever had dysuria, 60%, 9% and 22% respectively answered in the affirmative. Many girls were at risk of contracting *S. haematobium* as 65% of them reported having water contact. About 30% and 26% respectively of who reported red urine and dysuria had schistosomiasis ($P < 0.05$).

CONCLUSION The findings indicate at least one in three pupils may be infected with *S. haematobium* and the majority knew about bilharzia even though the accuracy of that knowledge was not ascertained. Many of them were exposed through water contact and red urine and dysuria were significantly ($P = 0.046$) associated with bilharzia.

P.1.4.5.007 (B)

Distance of river from school may be an indicator for *S. haematobium* infection amongst pupils from Ugu district in KwaZulu-Natal, South Africa

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BACKGROUND Water related activities may lead to high risk of infection in areas that are endemic for *S. haematobium*. Fetching water, washing clothes and fording rivers may be the most important water contact activities amongst teens and adults in rural areas, swimming and bathing remain important activities with respect to schistosomiasis infection.

AIM To determine if the distance between the rivers and the schools are significantly associated with schools prevalences of *S. haematobium*.

METHODS A cross sectional descriptive study was conducted in Ugu district, KwaZulu-Natal amongst female pupils aged 10–12 (N = 1057) from 18 randomly selected rural schools in areas with altitude below 300 m and consent was obtained from parents. Three urines, collected on consecutive days between 10 am and 2 pm, were obtained from each study participant and the urine centrifugation method was used for analysis.

RESULTS Overall prevalence of *S. haematobium* was found to be 28.1% (95% CI:25.0–31.0%) and mean intensity was 16.7 eggs/10 ml urine. Eleven schools were 0.7 kilometres (km) from nearest river and the rest were located further away, the most distant being 1.7 km. Six of 11 schools <0.7 km from river prevalences of *S. haematobium* infection ranged between 20% and 67% .. Five of seven schools furthest away had a schistosomiasis prevalence lower than 20%, the other two had prevalences of 36% and 46% respectively.

CONCLUSION Schools nearer to the rivers had higher *S. haematobium* infections indicating that learners may have easy recreational exposure to rivers putting them at higher risk of infection, although there is also the possibility that some infected pupils may have contacted the infection from streams nearer to their homes.

P.1.4.5.008 (B)

Estimating the cost of the mass treatment campaign for schistosomiasis in Ugu District, KwaZulu-Natal, 2012

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INTRODUCTION Schistosomiasis is a parasitic disease that affects developing countries; it has economic and public health significance. WHO recommends regular treatment for schistosomiasis in endemic areas and has that estimated that more than 5 million individuals require treatment in South Africa. The evidence shows that there is a need for implementation of a schistosomiasis Mass Treatment Campaign (MTC) but information about the cost of such a programme is unknown in South Africa.

MATERIALS AND METHODS A retrospective, cross sectional descriptive costing study for a schistosomiasis MTC was conducted, from a provider perspective. Costs were classified by cost inputs and activity. Financial records were reviewed for all 60 randomly selected schools participating in the MTC in Ugu

District, KwaZulu-Natal. An Excel database was used for data analysis. Preliminary Findings

Forty five percent (16 239/36202) of the children were treated. The total cost of the Mass treatment campaign was US$ 198.994. The unit cost per child treated was US$ 12.29. Had 75% of the children been treated the unit cost per child would have been US$ 7.3. The major cost drivers were praziquantel tablets (44%) and personnel (42%) followed by consumables (9%), transport (4%) and capital items (1%) respectively. The highest cost proportion by activity was Treatment costs (74%) followed by administration (19%), school visits (4%) and advocacy (3%) respectively.

CONCLUSIONS The praziquantel tablets and personnel contribute the highest costs. The South African Medicines Control Council does not accept WHO accredited medication; If free or cheap praziquantel is made available many more children could be treated. Furthermore, an increase in coverage rate would also make the operation cheaper. The integration of the programme with other health campaigns could also be cost saving. Health education and advocacy should be strengthened in order to increase the uptake of the Mass treatment Campaign.

P.1.4.5.009 (B)

Co-infection with *Schistosoma haematobium* and soil-transmitted helminths among school girls from rural Ugu district in southern KwaZulu-Natal, South Africa: a cross-sectional survey

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BACKGROUND Schistosomiasis and soil-transmitted helminthiasis (STH) are two of the most prevalent neglected tropical diseases. Association between the different helminths are found in other areas, but have not been investigated in South Africa. This study assesses the extent of co-infection between *S. haematobium*, *A. lumbricoides* and *T. trichiura* in rural Ugu district in KwaZulu-Natal (KZN) and seeks to explore if *S. haematobium* could serve as an indicator for soil-transmitted helminths.

MATERIAL AND METHODS One thousand and fifty-seven primary school girls between the ages 10 and 12 years were included from 18 randomly selected schools. Urine and stool samples were examined for the presence of eggs using the urine sedimentation technique for *S. haematobium* and the Kato Katz technique for soil-transmitted helminths. Person's chi-square test was used to calculate the association between the species based on prevalence and Likelihood ratio were used for calculation of associations between intensity groups.

RESULTS The prevalences for *S. haematobium*, *A. lumbricoides* and *T. trichiura* were found to be 37.4%, 25.3% and 27.2% respectively. The arithmetic mean (AM) of intensity for *S. haematobium* was 17.3 eggs/10 ml of urine [geometric mean (GM) = 1.64 eggs/10 ml]. For *T. trichiura* AM was 106.8 EPG (GM = 2.04 EPG). For *S. haematobium* and *T. trichiura* the majority of the infected girls had light infection. Most girls infected with *A. lumbricoides* had moderate/high infection. Significant association were found between *S. haematobium* and *A. lumbricoides* ($\chi^2 = 6.77$, $P = 0.009$), *S. haematobium* and *T. trichiura* ($\chi^2 = 25.37$, $P < 0.001$), and *A. lumbricoides* and *T. trichiura* ($\chi^2 = 131.45$, $P < 0.001$).

CONCLUSION This study demonstrates a highly significant association between schistosomiasis and *A. lumbricoides* and *T. trichiura* and concludes that *S. haematobium* may serve as an indicator for soil-transmitted helminths in this environment.

P.1.4.5.010 (B)

Urinary tract morbidity due to *S. haematobium* in patients attending Américo Boavida Hospital, Luanda, Angola

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INTODUCTION Chronic infections due to *Schistosoma haematobium* account for almost 40% of urinary tract abnormalities (UTA) in patients attending the Urology Service of the Américo Boavida Hospital. UTA's related to parasite were assessed by parasitology, ultrasonography (US), and if necessary by cystoscopy and histopathology. Furthermore, the accuracy of NMP22 test for early detection of bladder malignancy was investigated.

MATERIAL AND METHODS A prospective study was conducted in 104 patients (48 male; 56 female; 18–75 years, median 37). Urine was collected for parasitology, cytology and NMP22 tests and US examination of the urinary tract was performed in all subjects, without prior knowledge of the laboratory results. Patients with morphological bladder and renal lesions seen by US were submitted to cystoscopy and suspicious lesions were assessed by histopathology of biopsies. Statistical analysis was performed using SPSS software version 16.0.

RESULTS *S. haematobium* ova were found in 27% of the patients, the majority (18.3%) excreting low to moderate egg numbers (1–49 eggs/10 ml of urine). UTA's detected by US included hyperechogenicities of the bladder wall (55.8%), bladder masses (23.1%) and upper urinary tract obstruction (UTO) (54.8%). Cystoscopy performed in 74 patients revealed characteristic 'sandy patches' in 30.1% of cases; inflammatory cystitic changes containing *S. haematobium* ova in 55.4%, and masses in 42.5% of biopsies. Squamous cell carcinoma characteristic for schistosomiasis accounted for 70.9% of all bladder carcinomas diagnosed by the Urology Service. NMP22 test showed a slightly better correlation with histopathology than urine cytology and should therefore be assessed further for its potential as a field tool.

CONCLUSIONS Given the high frequency of serious UTA's due to schistosomiasis in the area, epidemiological investigations and interventions are warranted with the aim of preventing the occurrence of schistosomiasis related morbidity and to investigate reliable biomarkers for the early detection of schistosomiasis induced malignancies.

P.1.4.5.012 (B)

An association study of *Schistosoma haematobium* infection and bacteriuria in young South African females

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Introduction *Schistosoma haematobium* endemic areas are generally low-resource settings. Symptoms routinely used for cheap and easy proxy diagnosis of *S. haematobium* infection are haematuria, proteinuria, dysuria and pollakisuria, however these symptoms can also be invoked by urinary tract pathogens making misdiagnosis probable. It is possible that local lesions and inflammation in relation with schistosome egg granulomas alter the ability of bacteria to establish infection foci either positively or negatively. Therefore it is of interest to clarify whether bacteriuria is a confounder for common *S. haematobium* diagnostic tests.
Materials and Methods Urine samples from 1119 randomly selected female primary and high school students from Ugu district, KwaZulu-Natal, South Africa, were collected in a cross-sectional manner from May–August 2012. Samples were tested in the field for haematuria, proteinuria and leukocytes with Neotest4 dipsticks and 10 ml urine was added to 1 ml 5% formalin solution and preserved for later egg count microscopy. Furthermore samples were tested for bacteriuria with Uricult® dipslides (cut-off point ≥10^5 CFU/ml).
Results The mean overall prevalence for *S. haematobium* was 18.3% and 10% for asymptomatic bacteriuria. No association was found ($P = 0.172$, $n = 1039$) between *S. haematobium* infection and bacteriuria. Micro-haematuria was strongly positively associated with schistosomiasis ($P < 0.001$) whereas for bacteriuria only a tendency was observed ($P = 0.053$). Only the presence of leukocytes was positively associated with bacteriuria ($P = 0.002$) probably due to detection of neutrophils rather than eosinophils with the dipstick. As expected, intensity of *S. haematobium* infection was positively correlated with degree of micro-haematuria ($r = 0.505$; $P = 0.01$). Proteinuria was positively associated both with bacteriuria ($P = 0.002$) and *S. haematobium* ($P < 0.001$).
Conclusions No association between bacteriuria and urogenital schistosomiasis was demonstrated. Bacteriuria appears not to be a confounder for using micro-haematuria as diagnostic measure for *S. haematobium* infection in this sample. Further studies should be performed on symptomatic populations and in clean-catch urines.

1.4.6 Lymphatic filariasis

P.1.4.6.001 (A)

Lymphatic filariasis in Luangwa District, South-East Zambia

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Past case reports and recent data from LF mapping surveys indicate that LF occurs in Zambia, but no detailed studies have been carried out to document its epidemiology and health implications. The present study assessed infection, disease, transmission and human perception aspects of LF in an area of Luangwa District, South-East Zambia, as a background for planning and implementation of control. Populations in two neighbouring rural communities were registered and a questionnaire survey undertaken. Clinical examinations, and screening of blood for circulating filarial antigens (CFA; marker of adult worm infection) and antibodies to Bm14 antigen (marker of exposure to transmission), was carried out during daytime. Blood from CFA positive individuals was examined for microfilariae (mf) at night. Vector surveys were carried out in selected households, using light traps. A total of 985 individuals aged ¡Ý 1 year were registered. The prevalence of CFA increased with age from 1.2% in age group 1–14 years to 20.6% in age group 50+ years (overall prevalence 8.6%). Wuchereria bancrofti mf were identified in 10.9% of CFA positive individuals. Prevalence and mean intensity of Bm14 antibodies were much higher in individuals aged ¡Ý 30 years (57.2%; 0.594) than in younger individuals (19.3%; 0.241). Elephantiasis and hydrocele were well known clinical manifestations in the area, and had local names, but only one case of hydrocele was detected in the study population. Identified potential vectors were Anopheles funestus and *A. gambiae*. LF was therefore endemic in the study community, but infection prevalence was low. The much higher prevalence and intensity of Bm14 antibodies in the older than the younger part of the population, and the low overall rate of clinical manifestations, suggest that transmission in the area is on the decrease, perhaps because of intensive application of malaria control measures targeting the Anopheles vectors.

P.1.4.6.002 (A)

Lymphatic filariasis in the metropolis of Dar es Salaam, Tanzania

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The last decades have witnessed a considerable increase in urbanization in Sub-Saharan Africa, and more than 50% of the population is estimated to live in urban areas by 2040. Rapid growth of cities, combined with limited economic opportunities, often result in expansion of informal settlements and slums with favourable conditions for proliferation of vectors of lymphatic filariasis (LF). In Dar es Salaam, which has grown more than 30 times in population during the past 50 years (4.4 million inhabitants in 2012), historical surveys indicate high prevalences of LF. This study investigated the epidemiology of LF in the metropolis of Dar es Salaam, as a background for planning and implementation of control. Six sites with varying distance from the city centre (3–30 km) and covering different population densities, socioeconomic characteristics, and water, sewerage and sanitary facilities were selected for the study. Pupils from one public primary school at each site were screened for circulating filarial antigen (CFA; marker of adult worm infection) and antibodies to Bm14 (marker of exposure to transmission). Community members were examined for CFA, microfilariae and chronic manifestations. Structured questionnaires were administered to pupils and heads of community households, and vector surveys were carried out in selected households. Despite haphazard urbanisation, and generally poorly developed water, sewerage and sanitation facilities, the study indicated that a tremendous decrease in burden of LF had occurred. Contributing factors may be urban malaria control targeting Anopheles vectors, short survival time of the otherwise numerous Culex quinquefasciatus vectors in the urban environment, widespread use of bed nets and other mosquito proofing measures, and mass drug administra-

tion in 2006 and 2007. However, the burden of chronic LF disease manifestations was still high. The development has so far been promising, but continuing efforts are necessary to ensure elimination of LF as a public health problem.

P.1.4.6.003 (A)

Mapping lymphatic filariasias in Zambia using climatic-based species distribution modeling

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Mapping the spatial distribution of lymphatic filariasis is crucial for the planning and implementation of successful control. Recently there has been an increase in the application of the so-called climatic-based species distribution modeling methods, commonly applied within the field of ecology and conservation, for the mapping of a number of human pathogens, their vectors and hosts. Climate is considered to be the predominant range-determining mechanism for most species at large spatial scales, but ranges may also be controlled by other environmental or non-environmental factors. In the present study, based on national survey data on LF parasite prevalence, we employ species distribution modeling to make a first map of LF in Zambia. Specifically, we aim to identify the ecological correlates associated with different cut-offs of parasite prevalence (using remote sensing and other environmental data), and to use these correlates to develop maps that describe the presence of LF at two different prevalence levels (low and medium) at un-sampled areas across Zambia. The next logical step is to refine these maps using Bayesian geostatistical modelling to predict the exact prevalence at any given location in Zambia. Furthermore, potential interactions between LF and other mosquito-borne infections will be investigated, and geographical overlaps in spatial distributions and prevalence mapped. Maps of the distribution and risk of disease and areas where one or more parasitic infections overlap in space are useful from a control point of view, especially in light of recent years growing advocacy for integrated control of neglected tropical diseases.

1.4.7 Diagnosis of helminth infections

P.1.4.7.001 (A)

Mini-FLOTAC and Kato-Katz: helminth eggs watching on the shore of Lake Victoria

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Background One of the challenges for monitoring helminth control programmes is the lack of a copro-parasitological gold–standard diagnostic technique which combines good sensitivity with quantitative performance, low cost, and easy-to-learn technique. The aim of our study was to evaluate and compare, the WHO recommended quantitative diagnostic technique (Kato-Katz) and the Mini-FLOTAC, an innovative method based on floatation of helminths eggs with two different solutions (FS2 saturated sodium chloride; FS7 zinc sulphate) using a close system (fill-FLOTAC) with 5% fixative. The study was carried out in rural Tanzania, close to Lake Victoria, where the laboratory facilities are scarce, and the basic technique used in the local laboratory (direct smear) was taken as reference standard.
Results Two hundred and one children were screened for intestinal helminths and 91% were found positive. The agreement among the three techniques calculated with *k* Cohen coefficient was fairly good (k = 0.4), although the Mini-FLOTAC detected the highest number of hookworm infection (73%) with FS2, and of *S. mansoni* (49%) with FS7 followed by Kato-Katz (68% and 33%, respectively) and direct smear (21% and 4%, respectively). A good agreement was found between Mini-FLOTAC and Kato-Katz with FS7 (k = 0.76) for hookworm, and a fairly good one for *S. mansoni* diagnosis (k = 0.5). For both infections a poor agreement between the two quantitative techniques and the direct smear was found (k < 0.3). Kato-Katz diagnosed a higher number of eggs compared with the Mini-FLOTAC (P = 0.4)both for hookworm (455 vs. 424 mean EPG) and for *S. mansoni* (71 vs. 58 mean EPG).
Conclusions Mini-FLOTAC is a promising technique, comparable and sensitive as the Kato-Katz.. Comparative advantage is that Mini-FLOTAC comprises a close system that both protects the operators and allows subsequent examination of the preserved samples. Further studies are needed to validated the Mini-FLOTAC with other quantitative techniques (McMaster) and in settings where other soil-transmitted helminths are endemic.

P.1.4.7.002 (A)

Use of occult blood detection cards in the real time PCR-based diagnosis of *Schistosoma mansoni* infection

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Objective The need for accurate, highly sensitive diagnostic tools for Schistosomiasis has been emphasized. In the recent years molecular methods to diagnose *Schistosoma mansoni* and *S. heaematobium* in stool and urine samples have been developed. In order to obtain optimal field applicability the use of occult blood detection cards (fecal cards) for stool sampling seems a possible option. The purpose of this pilot study was to evaluate the sensitivity of PCR after storing stool samples on fecal cards.
Methods Stool specimens were collected in a highly endemic area for *S. mansoni* in Ethiopia and blinded after microscopic examination. Aliquots of fresh stool samples and corresponding stool smears on fecal cards were analyzed by real-time PCR.
Results Out of 100 stool samples 55 specimens were evaluated by PCR, 35 of which were positive and 20 negative by microscopic examination. When microscopy was used as diagnostic 'gold standard', the sensitivity of PCR on fresh stool was 94.3% (95%-CI: 86.6; 100) and on fecal cards 91.4% (95%-CI: 82.2; 100). There was no statistically significant difference between both PCRs (P = 1; Fisher exact).

CONCLUSION The use of fecal cards proofed to be a simple but useful method for stool collection and storing prior molecular analysis for *S. mansoni*. This technique may be a valuable approach for large scale surveillance and post treatment assessments.

P.1.4.7.003 (A)

Usefulness of *Strongyloides stercoralis* serology in the management of patients with eosinophilia

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INTRODUCTION Diagnosis of strongyloidiasis is based on larvae detection in the stools. However, in chronic asymptomatic patients, the intestinal worm load is very low and the output of larvae is irregular, hence the sensitivity of direct observation of larvae decreases considerably. The aim of this study is to evaluate the usefulness of Strongyloides stercoralis serology for the diagnosis of strongyloidiasis in patients presenting eosinophilia and its role in the follow-up after treatment.
MATERIAL AND METHODS Prospective observational study performed at the Infectious Diseases Department of the Hospital Universitari Vall d'Hebron, Barcelona (Spain). Patients with eosinophilia (eosinophil cell count ≥500 cells/mm³ and/or a percentage ≥7%) attended from January 2010 to December 2012 were included. Strongyloides stercoralis serology, microscopic investigation of stool samples from three different days and faecal culture were performed in all patients. Ivermectin 200 μg/kg/day for two days was offered to all patients with confirmed diagnosis or suspected diagnosis (presence of eosinophilia, positive Strongyloides stercoralis serology and the absence of other causes of eosinophilia). Follow-up of treated patients were performed after 6 months of completion of treatment.
RESULTS One hundred and forty-seven patients were included, the median age was 35 (range 18–76) years and 75 (51%) patients were male. Twenty-eight (19%) patients had HIV infection. 89 (60.5%) patients had a positive serology and larvae in stools were detected in 15 (10.2%) patients. From these 89 patients with positive serology, nine had other causes of eosinophilia, 48 patients were lost during follow-up or didn't reach 6 months of follow-up when the study finished. In patients who completed the study protocol we observed a reduction in both the eosinophil cell count and the optical density of serology (table).
CONCLUSIONS Strongyloides stercoralis serology is more sensitive than direct techniques for the diagnosis of strongyloidiasis in patients presenting eosinophilia, and is useful for the post-treatment follow-up.

Table 1 Baseline and 6 months post-treatment results of patients who completed the study protocol

	Baseline (n = 32)	6 months post-treatment (n = 32)
Absolute eosinophil cell count (cells/mm³)	1050 (575–4000)	200 (100–700)
Relative eosinophil cell count (%)	15.7 (8.2–38.5)	3.6 (0.8–9.6)
Positive *Strongyloides stercoralis* serology	32/32 (100%)	21/32 (65.6%)
Optical density of serology	7.01 (1.65–18.60)	1.38 (0.16–7.78)
Detection of larvae in stools	4/32 (12.5%)	0/32 (0%)

Data are reported as number (%) of patients or median value (range).

P.1.4.7.004 (A)

Diagnosis of active *Schistosoma mansoni* infection by molecular and immunological methods in school children living in low endemicity areas

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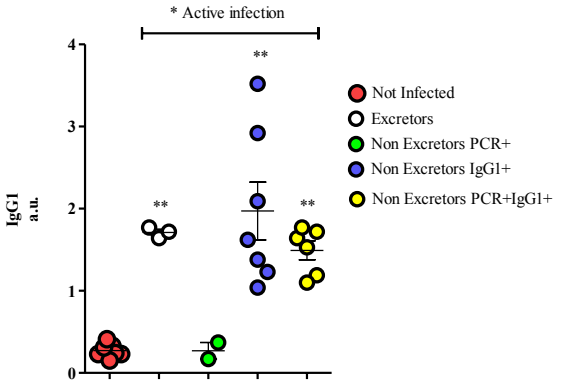
INTRODUCTION In low endemicity areas, diagnosis of active Schistosoma infection is still a challenge. Mostly, infected individuals present light infections without egg excretion, which are undetectable by microscopy. In surveillance settings, school children represent a sentinel population for determining infection endemicity. However, despite failures in infection detection, coproscopy remains the solely reference test. Although molecular and immunological methods are sensitive for diagnosis, few groups use both methods for surveillance. This study aims to determine the accuracy of molecular and immunological methods to diagnosis active infection in school children population.
MATERIAL AND METHODS Study population of 108 individuals from a rural area of Rio de Janeiro comprised 23 school children at ages of 8–19 years old, being 52.2% females. After informed consent (FIOCRUZ Ethics Committee, CAAE-0038.0.011.000-0) obtained from parents, enrolled individuals provided fecal and blood samples. *S. mansoni* infection was determined by Kato-Katz (two slides/stool sample, K-K), specific serum IgG1 anti-adult worm (SMMA) levels measured by ELISA and DNA amplification by Real-Time PCR in fecal samples. Statistical analysis was performed by ANOVA by using GraphPad Prism 5.00 (San Diego, CA).
RESULTS Active Schistosoma infection was confirmed by K-K and/or IgG1 and/or PCR positivity in 15/23 individuals. Egg excretion was detected in 3/15 infected individuals (K-K+PCR+IgG1+). In non egg-excretors, five had PCR+IgG1+ except one individual. In seven out of 12 non excretors, IgG1 was reactive in the absence of DNA amplification. In this group, four individuals had no history of PZQ use suggesting recent infection. Levels of IgG1 were significantly elevated in infected (excretors/non excretors) versus not infected individuals (mean arbitrary units (a.u.) ±SD: 1.71 ± 0.07 a.u. and 1.55 ± 0.85 a.u. × 0.24 ± 0.08 a.u., respectively $P < 0.01$).
CONCLUSIONS Molecular and immunological methods are reliable tools for diagnosis of active Schistosoma infection in school children living in areas of low endemicity.

Total of 23 school aged children were investigated. Specific IgG1 anti- *S. mansoni* adult worm antigen levels were determined by ELISA and results expressed by arbitrary units (a.u.). Statistical analysis was performed by One –way ANOVA with Dunn's post-test (GraphPad Prism 5.00, CA). *$P = 0.018$ (for the difference between active infection versus not infected group). *$P < 0.05$ (for the difference between excretors versus not infected; non excretors versus not infected). Excre-

tors = K−K+/IgG1+/PCR+; Non Excretors PCR+ IgG1+=K−K−/IgG1+/PCR+; Non Excretors IgG1+=K−K−/IgG1+/PCR−; Non Excretors PCR+ = K−K−/IgG1−/PCR +; Not Infected = K−K−/IgG1−/PCR−.



IgG1 reactivity and/or PCR positivity without egg excretion characterizes active infection in non egg-excretors in 8-19-year-old children living in areas of low endemicity.

P.1.4.7.005 (A)

Serological diagnosis of Strongyloides infection in migrants with eosinophilia in Barcelona

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INTRODUCTION Current clinical guidelines include the Strongyloides serology for the study of asymptomatic eosinophilia in migrant people. The aim of this study is to evaluate the usefulness of Strongyloides serology for etiologic diagnosis of eosinophilia in migrants living in Barcelona.
MATERIAL AND METHODS A pilot study was carried out in two Primary Health Care centers in l'Hospitalet de Llobregat (Barcelona), which attend approximately 20% of migrant population, during the period April–August 2011. Strongyloides serology was performed in patients who presented eosinophilia. We assayed the presence of Strongyloides IgG antibodies by ELISA (Strongyloides serum Microwell ELISA, IVC®). Absorbance >0.2 OD was considered positive. Eosinophilia levels were classified as: mild (>0.45 $\times$ 10^9 eos/l), moderate (1.5–3.0 $\times$ 10^9 eos) and high (>3 $\times$ 10^9 eos/l).
RESULTS A total of 173 patients were studied, 22 (12.7%) of whom were <15 years old. Strongyloides serology was positive in 36 (20.8%) patients, all of them were adults. Regarding to the geographical areas of origin, 71.1% came from Latin America, 12.1% from Indo-Pakistan, 8% from Maghreb and 4% from Saharan Africa. Out of 36 patients with positive serology, 35 were from Latin America, and one from Nepal. We found no positive Strongyloides serology among patients from Indo-Pakistan, Maghreb or Saharan Africa. This fact may be influenced by the small sample size. Considering the level of eosinophilia, 160 patients presented mild eosinophilia of whom 28 (17.5%) had positive Strongyloides serology. The remaining 13 patients showed moderate eosinophilia, of whom 8 (61.5%) had positive serology. No patient was found to have high grade of eosinophilia.
CONCLUSIONS Strongyloides stercoralis is an important cause of eosinophilia in Latin American population living in Barcelona. More than half of moderate eosinophilia cases in Latin Americans were caused by this helmith. In our opinion eosinophilia study should include Strongyloides serology in these populations.

1.4.8 Progress towards alternative anhelminthic drugs

P.1.4.8.001 (A)

Anthelmintic activities of a plant-derived natural product

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INTRODUCTION Schistosomiasis control is heavily reliant on a single drug, praziquantel (PZQ). With no vaccine on the immediate horizon, and the ever-present spectre of PZQ-resistant schistosomes developing in communities undergoing mass drug administration (MDA) programmes, identifying next generation anthelmintics is urgently needed for sustaining deworming initiatives into the future. Towards this end, and due to the well-characterised therapeutic success of artemisinin, we have begun investigating the anthelmintic activities of natural products derived from temperate plants. Here, we demonstrate that, amongst a library of novel chemical entities (NCEs), a single compound displayed both anti-schistosomal and anti-fasciolicidal activity during *in vitro*, whole organism assays.
MATERIALS AND METHODS *Schistosoma mansoni* schistosomula viability was assessed by the helminth fluorescent bioassay, adult schistosome viability was assessed by a WHO-TDR developed phenotypic matrix and Fasciola hepatica newly excysted juvenile (NEJ) viability was evaluated by morphology and motility measures. NCE-mediated adult schistosome phenotypic alterations were also examined by scanning electron microscopy and fluorescent confocal imaging. General NCE-mediated cytotoxicity was measured on HepG2 cells via a CellTiter-Glo® luminescent assay.
RESULTS This NCE displayed an LD50 = 20 μM against schistosomula, an EC50 = 2.5 μM against *F. hepatica* NEJs and caused severe phenotypic alterations in adult schistosomes (between 10 and 100 μM). These adult schistosome alterations included tegumental disruptions, tubercle abnormalities and irregular oocyte architecture. Reassuringly, this compound displayed low cytotoxicity (50 μM) against a human liver cell line (HepG2).
CONCLUSION In light of the selective and pan-trematode activity (against both blood and liver flukes) of this natural product, further experiments are on-going to detail additional anthelmintic properties in both *in vitro* and *in vivo* models.

1.4.9 Trypanosomiasis and leishmaniasis

P.1.4.9.001 (B)

Prevalence of *Trypanosoma cruzi* infection among people aged 15–89 years inhabiting the Department of Casanare (Colombia)

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INTRODUCTION *T. cruzi* Infection usually remains undetected for decades and an important proportion of these patients eventually progress to cardiomyopathy and hearth failure. Recognition of infected people is important to avoid disease spreading through transfusions and transplantations. Here we aimed to describe the prevalence of the infection by *T. cruzi* and its risk factors in a region of Colombia previously considered at a high risk for the infection because of the presence of infected insect vectors in housings.
MATERIALS AND METHODS A cross-sectional study was conducted in subjects from five municipalities, recruited in urban and rural locations, distributed by gender according to the demographic information available. Socio-demographic information, history of potential exposure to insect vectors, blood donating, as well as symptoms suggesting cardiac disease were collected using a questionnaire. After giving written informed consent, blood specimens were obtained from 486 people to determine the serologic evidence of past exposure to *T. cruzi*. Infection was diagnosed when two different serologic tests (ELISA and IHA) were positive.
RESULTS The seroprevalence of antibodies against *T. cruzi* was 16.91% considering an estimated population of 44 355, aged between 15 and 89 years (95%CI: 13.72–20.01), with a confidence level of 95%. The factors significantly associated with the infection were: (i) Housing materials like vegetable material, adobe or unfinished brick walls; (ii) The fact of having previous tests for Chagas disease (regardless of the result). In addition, it is of note that the mean ages among infected and not infected participants were significantly different (99% CI of differences: -12.28 to -2.784, $P \leq 0.0001$ in two-tailed unpaired *t*-test). Among the studied municipalities, the one with the highest frequency of *T. cruzi* infection was Nunchia, with 31.15% of the surveyed subjects.
CONCLUSIONS *T. cruzi* infection is highly prevalent in the region of Casanare, in Colombia.

P.1.4.9.002 (B)

Heart transplant in a patient with HIV-Chagas Disease coinfection. First case report

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BRIEF INTRODUCTION Cases of reactivation of Chagas Disease (CD) in patients infected with human immunodeficiency virus (HIV) have already been reported. Both infections can cause progressive damage of target organs, particularly the heart. Although solid organ transplantation is an accepted option for both patients with HIV and for those with CD, in literature there is no mention of transplant in co-infected subjects. We describe the first case of a patient with CD and HIV co-infection, who successfully received heart transplant. Case Description
A 42-year-old Brazilian was admitted to hospital for heart failure, with left ventricular ejection fraction (LVEF) of 25%. He also presented generalized lymphadenopathy and low white cell count, so a HIV test was done and found positive (at baseline, viral load: 53 325 copies/ml and CD4 cells count: 30 cells/mm^3). The patient was placed on highly active anti-retroviral therapy (HAART) plus cotrimoxazole prophylaxis. Taking into account the patient's epidemiological background, the screening tests for *Trypanosoma cruzi* were done and resulted positive. Moreover, a reactivation of Chagas Disease was demonstrated through micro hematocrit tube technique (MHT), which demonstrated the presence of trypomastigotes of *T. cruzi* in the peripheral circulation. A course of benznidazole (5 mg/kg/day) was promptly initiated and appeared well tolerated. Unfortunately patient's cardiac dysfunction further worsened (LVEF reached 14%), despite inotropic support. Considering the patient's age and the good response to the antiretroviral and antitrypanosomal therapy (which permitted to reach undetectable HIV load and *T. cruzi* parasitemia), evaluation for heart transplant was sought. The patient was successfully transplanted, benznidazole was discontinued before transplant, after persistent demonstration of negative *T. cruzi* parasitemia and successful immune reconstitution. *T. cruzi* PCR follow-up was constantly negative in the post-transplant phase. Currently, 19 months after transplant, the patient is in good health conditions.
CONCLUSIONS Organ transplantation may be an option for HIV- CD coinfected patients with end-stage organ failure.

P.1.4.9.003 (B)

Arthritis during treatment with Benznidazole

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INTRODUCTION Chagas disease is endemic in Latin America and an emerging disease in non-endemic countries due to the recent trends in migration. Benznidazole is the most commonly used drug for the etiological treatment. Patients treated with Benznidazole suffer frequent adverse drug reactions and whereas arthromyalgia is common arthritis has been reported as a very rare symptom. The objective of this study is to describe the importance and characteristics of arthritis as an adverse reaction of Benznidazole.
METHODS We performed a retrospective cohort study of 178 patients attending to the Tropical Medicine Unit in the Hospital Clinic of Barcelona and initiating treatment with Benznidazole (5 mg/kg/day for 60 days) from June 2009 to June 2011.
RESULTS One hundred and seventy-eight patients in chronic phase were treated with Benznidazole. Most of them were from Bolivia, women (75.8%), age ranged from 25 to 43. Treatment was interrupted in 45 patients (25.3%), five of them (11.1%) due to arthritis. Another case of arthritis was diagnosed 2 days after treatment was stopped due to dermatological adverse event. Arthritis developed in the six patients (3.4% of all treated) between day 25 and 42 after starting treatment. One case had additive arthritis, three oligoarthritis and two polyarthritis. In five of them was asymmetric. Autoimmunity test were normal in five out of six. All affected patients were treated with non-

steroidal anti-inflammatory drugs and steroids and the symptoms were solved in 7–62 days with no relapse or chronicity. Conclusions We have observed that arthritis is more frequent than previously described in patients treated with Benznidazole and it can be the main reason for discontinue treatment. We did not find an established pattern of joint involvement and no patient developed chronic symptoms afterwards. It appears that Benzidazole can cause arthritis but there is no evidence that it could trigger a chronic autoimmune disease.

P.1.4.9.005 (B)

Performance evaluation of enzyme linked immunosorbent assay and lineblot for serological diagnosis of Chagas (*Trypanosoma cruzi*) disease

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Chagas disease, caused by infection with the protozoan parasite *Trypanosoma cruzi*, affects 8–11 million individuals worldwide. It is endemic from the south of the US to Central America and South America. The disease is commonly transmitted by an insect vector but may also be spread through blood transfusion and organ transplantation, ingestion of food contaminated with parasites and from a mother to her fetus. Without specific treatment, the mortality rate among such children is high. Early diagnosis is essential so that etiological treatment can be administered. Screening of donated blood, blood components, and solid organ donors, as well as donors of cells, tissues, and cell and tissue products for *T. cruzi* is mandated in all Chagas-endemic countries and has been implemented. Due to travelling to endemic regions and immigration from endemic regions Chagas can be found also in Europe. Serodiagnosis of new borns is often difficult due to their altered immune system and maternal immunoglobulin still present in the blood. Here we describe a new Novalisa ELISA and lineblot for diagnosis of Chagas disease. These test systems are taking advantage of the chimeric multiepitope antigen TcF (IDRI). The test was evaluated in endemic countries like Colombia and Guatemala with chronic patients (symptomatic and asymptomatic), pregnant women and newborns and a Chagas negative control group. Overall performance of both testsystems, ELISA and blot, was excellent. In addition both new testsystems were the only one who could reliably detect congenital transmission of Chagas from a mother to the child, making it essential for screening of new borns in endemic countries to provide treatment as early as possible. In our hands NovaTec Immundiagnostica ELISA and blots can reveal positive patients that currently used in house tests can not detect. Results were confirmed by qRT-PCR and compared with results of other commercial kits.

P.1.4.9.006 (B)

In vitro screening of synthetic compounds for anti-trypanosomal activity and cytotoxicity to human MRC5 cells

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There are currently only four drugs approved for treating Human African Trypanosomiasis (HAT) caused by *Trypanosma brucei* rhodesiense or *T. b. gambiense*. These include suramin, pentamidine, melarsoprol and eflornithine. In Zambia, HAT is caused by *T. b. rhodesiense* and effective drugs are pentamidine and suramin for the early stage and only merlasoprol for the late stage of infection. The disadvantages of these drugs are that (i) they are not orally bioavailable, therefore administration needs hospitalization, (ii) their effectiveness varies with stage of infection, (iii) they have serious adverse effects. It is therefore, necessary to find new drugs with fewer disadvantages for effective management of HAT. The objective of this study was to determine the *in vitro* activity against *T. b. rhodesiense* and the cytotoxicity to human MRC5 cells of selected compounds. Several classes of synthetic compounds were subjected to primary screening for *in vitro* activity against *T. b. rhodesiense* resulting in 14 that had good anti-trypanosomal activity. The 14 compounds were subsequently screened for cytotoxic effects on human MRC5 cells and seven compounds had good selectivity indices which were >50. Preliminary results show that the IC50 values against *T. b. rhodesiense* for the seven compounds were as follows: TROH34 – 2.83 mM; TRO35 – 2.96 mM; TROH32 – 3.13 mM; TROH30 – 3.21 mM; TROH31 – 3.95 mM; MHOH116 – 4.13 mM and VMOH25 – 4.6 mM, compared to 0.055 mM for pentamidine. The IC50 value against MRC5 cells for all the seven test compounds was >250 mM compared to >10 mM for pentamidine. The selectivity indices (SI) were as follows: TROH34 – >88.34; TROH35 – >84.46; TROH32 – >79.87; TROH30 – >77.88; TROH31 – >63.29; MHOH116 – >60.53 and VMOH25 – >54.34, compared to >181.82 for pentamidine. These results highlight some new classes of synthetic compounds that have promising activity against *T. b. rhodesiense* which require further investigations.

P.1.4.9.007 (B)

New modified phthalazine derivatives decrease specifically intracellular replication of *Trypanosoma cruzi in vitro* and *in vivo*: a first step in the development of new drugs

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Trypanosoma cruzi is responsible for Chagas disease which is a potentially fatal considerable endemic health problem in Latin America due to inadequate therapy and the lack of an effective vaccine [1]. The only drugs currently used worldwide for the treatment of Chagas disease are nifurtimox and benznidazole. However, they present significant side-effects and limited efficacy, especially in the chronic phase of the disease [2]. A series of new phthalazine derivatives containing side-chains ended with imidazole units attached to the pyridazine ring and nitro or

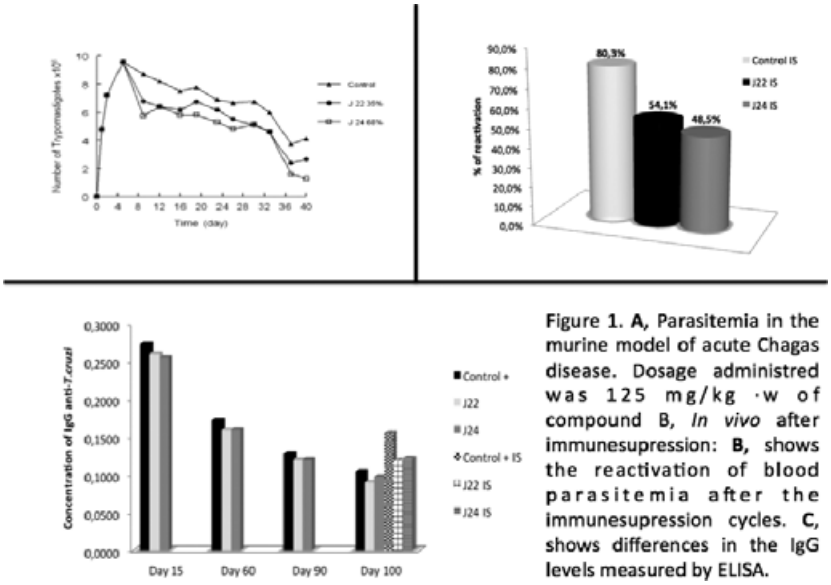

Figure 1. A, Parasitemia in the murine model of acute Chagas disease. Dosage administred was 125 mg/kg ·w of compound B, *In vivo* after immunesupression: **B,** shows the reactivation of blood parasitemia after the immunesupression cycles. **C,** shows differences in the IgG levels measured by ELISA.

hydroxyl substituents attached to the benzene ring were prepared. All *in vitro* and *in vivo* assays were performed according to the methodology previously established by us [3]. Compounds J22 and J24 were more active *in vitro* against *T. cruzi* and less toxic against Vero cells than the rest of analogues and the reference drug benznidazole (Table 1). The amastigote forms were found the most sensitive form for the drugs. *In vivo* tests on the acute phase of Chagas disease gave parasitemia decreasing values reaching 68% for J24 at day 40 post-infection (Fig. 1A) and the reactivation of parasitemia was found to be under 50% for J24 treated mice in the chronic phase after immunosuppression (Fig. 1B) and IgG levels were keept in those which were treated (Fig. 1C). The cure after the experiment (absence of parasites in the target organ) was found to be 33% and no evidence of toxicity in the biochemical profiles of kidney and liver was found. Attending to the mechanism of action, it was shown that compounds J22 and J24 highly inhibited the antioxidant parasite enzyme Fe-SOD which is one of the more important mechanism of escaping from the host defense. In conclusion compound J24 would be ready to continue to a clinical stage of testing.

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Figure 1 (A) Parasitemia in the murine model of acute Chagas disease. Dosage administred was 125 mg/kg w of compound (B) *In vivo* after immunesuppression: (B) shows the reactivation of blood parasitemia after the immunesuppression cycles. (C) shows differences in the IgG levels measured by ELISA.

P.1.4.9.008 (B)

Hospital discharges as a sentinel point of Chagas disease epidemiology in Ecuador

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BACKGROUND Official reports of Chagas disease in Ecuador are based in serological surveys as well as on occasional reports of acute or chronic cases. This could hide severe cases arriving at hospitals with a manifest disease. Hospital discharges could be an interesting observation point to know how big the burden of this disease is, as well as its demographic distribution.
METHODS Databases of hospital discharges of the National Institute of Statistics of Ecuador from 2000 to 2010 were examined. All records with a primary diagnosis matching with one of the ICD-10 codes B57, I41.2 or I98.1, corresponding to different forms of Chagas disease, were selected. Sex, age, residence and ICD-10 code were summarized.
RESULTS During the 10 years period 231 hospital discharges matched with one of the Chagas related codes, of which 18 died. Males accounted for 142 (64.5%) records. Ten records had <1 year old. Median of age among the 221 records having 1 or more years old was 64 (IQR: 44–75). ICD-10 codes B57.2 and B57.3 accounted for 192 (83.1%) cases. El Oro province located at the southwest accounted for 92 cases with an incidence rate of 1.63 discharges per 100 000 person-years. Sucumbíos, Loja and Morona provinces, located at the southeast and Amazonian region had an incidence rate of 0.32, 0.30 and 0.30 discharges per 100 000 person-years, respectively. Other provinces with cases were Guayas, Manabi, Pichincha, Los Rios, Orellana, Azuay, Esmeraldas, Cañar, Pastaza, Carchi, Bolivar and Chimborazo.

Conclusions Hospital discharges contributes to understand the epidemiology of Chagas disease. In Ecuador, southern and Amazonian provinces had the highest incidence rate. These findings underline the need of an updated evaluation of the problem in Ecuador.

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P.1.4.9.009 (B)

Overcoming the challenges of setting-up clinical trial sites in remote African rural settings

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Brief Introduction DNDiFEX004 is a phase 2/3 non-inferiority clinical trial to assess fexinidazole as a new oral treatment for human African trypanosomiasis (HAT) versus NECT (Nifurtimox Eflornitine combination) as the reference treatment). Several trial centres where selected in the two countries with the highest HAT prevalence.
Material and Methods Study documentation was submitted to an international ethics committee, as well as to local and MSF ethics committees for approval. Under the aegis of the HAT Platform, initial review of the disease epidemiological profile was followed by a key informant meeting with national programme experts of the affected countries in order to identify possible sites. Those were classified according to prevalence of the disease, available human resources, infrastructure and accessibility. A site assessment visit was performed together by DNDi and Swiss TPH to confirm the platform's recommendations.
Results Five centres in referral hospitals in the Democratic Republic of Congo and one in the Central African Republic were selected. Internet access had to be set up in four out of six sites to enable the transmission of electronic case report forms. Buildings were refurbished and emergency medical equipment supplied; solar energy equipment or generators were installed so a regular electricity supply would guarantee the cold chain needed for transport, storage and analysis of tissue samples. In addition, technical equipment was adapted for field use and local personnel were trained in GCP guidelines, trial procedures, as well as HAT diagnostic techniques and treatment procedures.
Conclusions Close collaboration with local and international experts, and with national programmes, as well as thorough training of staff and improved infrastructure at the study sites allowed successful trial initiation in difficult settings.

P.1.4.9.010 (B)

'Tsetse traps and why do we fear them?' Community-centred tsetse control in Uganda

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There is renewed vigour in efforts to eliminate neglected tropical diseases including sleeping sickness (human African trypanosomiasis). Towards this end, efforts are being made to develop more cost-effective methods of tsetse control. In the West Nile region of Uganda, novel designs of insecticide-treated target are being deployed over an area of ~500 km^2. The operational area covers villages where tsetse control has not been conducted previously. The effectiveness of the targets will depend, in part, on their acceptance by the local community. Accordingly, we assessed knowledge, perceptions and acceptance towards targets in villages where targets and traps had or had not been used previously. Sixteen Focus group discussions with male and female participants were conducted in eight villages across Arua District. Discussions were audio recorded, transcribed and translated. We used thematic analysis to compare the views of both groups and identify salient themes. Preliminary findings indicate that despite the villages being <10 km apart, community members perceived deployed baits very differently. Villagers who had never seen traps before expressed fear, anxiety and panic when they first encountered them. This was related to associations with witchcraft and 'river ghosts' which are linked with physical or mental illness, death and misfortune. By contrast, villagers living in areas where traps had been used previously had positive attitudes towards them and were fully aware of their purpose and benefits. The latter group reported that they had similar negative perceptions when tsetse control interventions first started a decade ago. Our results suggest that despite their apparent proximity, acceptance of traps varies markedly between villages and this is related to the duration of experience with tsetse control programs. The success of community-based interventions against tsetse will therefore depend on early sensitisation campaigns that reach all communities, especially those living in the areas new to such interventions.

P.1.4.9.011 (B)

Trypanosoma cruzi infection in a non-endemic country

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Introduction Chagas disease was considered a disease linked to rural areas of Latin America. However, it has been increasingly diagnosed in non-endemic countries in the last decades due to migrant flows. The aim of this study is to evaluate epidemiological and clinical characteristics of *Trypanosoma cruzi* infected patients in a non-endemic country.
Material and Methods This is a prospective observational study performed at the Tropical Medicine Units of International Health Program of the Catalan Health Institute, Barcelona (PROSICS Barcelona, Spain). All patients with Chagas disease (diagnosis was based on two different ELISA serologic tests) attended at PROSICS Barcelona from June 2007 to May 2012 were included. Clinical and epidemiological data were collected: age, gender, country of origin, time since arrival to our country, clinical symptoms, comorbidities, visceral involvement study and completion of treatment.
Results During this 5-year period, 1274 patients were included, mean age of patients was 37.7 (range 18–81) years, 860 (67.5%) were women and most of them came from Bolivia (97%). Thirteen patients had comorbidities conditioning immunosuppression. Cardiac involvement prevalence was 16.9% (190 out of 1125 patients), lower than in studies performed in endemic areas (20–60%). Digestive involvement prevalence was

14.8% (154 out of 1041 patients); prevalence of gastrointestinal involvement was very different among studies because of different diagnostic tools and strategies used. Six hundred and thirty-six out of 1245 (51.1%) patients received treatment during the study period; treatment was definitely stopped in 87 (13.7%) patients due to toxicity.
CONCLUSIONS Chagas disease in non-endemic countries becomes a new disease, with lower morbidity and affecting younger patients, though with an increasing risk of coexistence with immunosuppressant conditions.

P.1.4.9.012 (B)

Chagas' desease: dificulty of effectiveness evaluation

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INTRODUCTION Compliance, monitoring and effectiveness evaluation of the treatment with benznidazole in patients with Chagas' disease.
MATERIAL AND METHODS Observational retrospective study of the patients treated between 2010 and 2011 and a following up period till 2013. Medical histories were reviewed, recording following data: demographics, disease state, compliance, side effects and analytic evolution.
RESULTS During the analyzed period, 22 patients (20 adults and two pediatrics) were prescribed benznidazol following OMS criteria. Average age was 37.1 ± 8.7 years in adults group, 70% were women. At the beginning of treatment 50% of patients had Chagas' cardiomyopathy, 25% gastrointestinal involvement and 5% both. Only 60% ended the treatment correctly, side effects were the only cause of treatment discontinuation and appeared in a 57% of the patients: 73% were dermatologic, 18% neurologic and 45% other as temperature, nausea, vomiting. No patient achieved negative serologic tests after treatment (IFI and EIA). Nowadays, medical following is possible in 35% of patients, 65% returned to their countries. The pediatric's group average age was 48 days, both male congenital infected. The only one that had medical monitoring didn't receive treatment and 3 months later all tests were negative (IFI, EIA y PCR).
CONCLUSIONS The 80% of the patients treated in our hospital presented cardiac and/or gastrointestinal involvement. Although benznidazole is probably the best tolerated drug for Chagas' disease treatment, incidence of side effects is high(50%) causing 35% of discontinuation; we think close monitoring is necessary for a better side effects management. Serological conversion timeline, 8–10 years average, and low taxes of cure, contribute to patient following loss making difficult the cost-effectiveness evaluation. In pregnant women narrow down and treatment must be prioritized in order to prevent newborns to acquire congenital Chagas' infection. Chagas' disease is an emergent health problem in Spain because of the immigration of endemic areas.

P.1.4.9.013 (B)

Benznidazol and triazol Research group for nanomedicine and innovation on Chagas disease (BERENICE). A new treatment for Chagas disease

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INTRODUCTION Chagas disease is an important health problem in Latin America and a challenge in non endemic countries. Although Chagas disease has been identified and described for more than 100 years, the therapeutic alternatives are limited. Benznidazole and nifurtimox, the only two drugs available for treatment, have frequent side effects, especially in adults, requiring discontinuation in up to 10% of patients.
MATERIAL AND METHODS Nanotechnology will be used for the encapsulation of benznidazole. This new approach will allow a release of medication directly into the intracellular space, therefore increasing tissue drug concentration and avoiding side effects. A better toxic profile will be achieved because of the reduced amount of benznidazole used. BERENICE Consortium has been created to carry out this task as a European research network coordinated by Vall d'Hebron University Hospital. BERENICE brings together eight European and Latin American partners: Nanomol Group of the Materials Science Institute of Barcelona-Spanish Scientific Research Council (ICMAB-CSIC), NanoBioCel Group of the University of Basque Country (UPV/EHU), Instituto de Higiene e Medicina Tropica de Lisboa, Parasitology Laboratory of the Faculty of Pharmacy at the University of Barcelona, Fundacao Oswaldo Cruz, National Institute of Parasotology Argentina Dr. Mario Fatala Chaben, and two pharmaceutical companies, ELEA and PRAXIS. Starting in September 2012, this 5-year project is supported by the European Commission under the Health Innovation Work Programme of the 7th Framework Programme. Further information at http://berenice-project.eu/.
RESULTS A safer and optimized drug delivery through nanotechnologies will be achieved. The toxic profile of the main current treatment, benznidazole will be improved. The trypanocidal activity of the new drug, alone and in combination will be tested *in vitro*, *in vivo* and in humans.
CONCLUSIONS The main objective of BERENICE project is to obtain a more effective, better tolerated and low-cost formulation of a drug with trypanocidal activity.

Abstracts of the 8th ECTMIH and 5th CSBSP

P.1.4.9.014 (B)

The role of molecular techniques in the management of chronic Chagas disease patients in a non-endemic country

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Introduction Chagas disease (CD) is a protozoan infection caused by *Trypanosoma cruzi* with an estimated prevalence of 8 million people. The chronic form of CD is characterized by the absence of symptoms and low parasitaemia, but 20–30% of patients develop the symptomatic form of chronic CD, with cardiac and/or gastrointestinal disorders. The aim of this study is to evaluate the correlation of real-time PCR (RT-PCR) and clinical and epidemiological features in patients with chronic CD in a non-endemic country.
Material and Methods All adults patients (age over 18 years) with chronic CD (two positive different serologic tests) and RT-PCR performed previously to receive specific treatment, attended at PROSICS Barcelona from June 2007 to May 2012, were included. for the RT-PCR technique any volume of blood was accepted. Samples were pre-treated with guanidine hydrochloride, and TCZ1/TCZ2 primers and TZ3 probe from satellite sequence were used in a TaqMan-based assay. Cardiac assessment was performed through electrocardiogram, chest x-ray and echocardiogram; digestive involvement was performed through barium enema and esophagogram.
Results Five hundred and fifty patients were included and RT-PCR was positive in 223 (40.5%) of them. No statistically significant difference were found between RT-PCR positive and RT-PCR negative patients in relation to epidemiological features: proportion of women (39% vs. 61%), age <40 years (40% vs. 60%) and proportion of patients who arrived to our country more than 3 years ago (41% vs. 59%). No differences in RT-PCR sensitivity were observed between symptomatic (43.7%) and indeterminate (39.3%) chronic CD, prevalence of cardiac involvement (43.6% vs. 56.4%) and prevalence of gastrointestinal involvement (44.3% vs. 55.7%).
Conclusions RT-PCR allows detection of patients with parasitaemia in the chronic stage of CD. Nevertheless, no differences were observed between RT-PCR positive and negative patients in relation to epidemiological data and clinical features.

P.1.4.9.015 (B)

Chagas cardiomyopathy: experience from a non-endemic country

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Background Chagas disease has become a new challenge for european countries. In the last decade migratory flows from Latin America have spectacularly increased, as so patients with Chagas disease. Chagas disease produces a chronic damage to the heart, leading to heart failure or rhythm disturbances. Development of cardiac alterations varies from endemic and non-endemic countries. To assess the cardiac involvement of a chagasic non-endemic population we conducted this study.
Methods Patients with Chagas disease, attended at Hospital Vall d'Hebron Barcelona (Spain), with electrocardiogram and echocardiogram records were selected. Two hundred and eighty-four out of 600 fulfilled inclusion criteria. Epidemiological and clinical variables were recorded and analyzed. PASW 18.0.0 was used to analyzed the data.
Results From the 284 patients included, 82.5% were from Bolivia, mean age was 38 years (19–81). Kuschnir classification found no signs of cardiomyopathy in 77.6% of the patients. Variables from the electrocardiogram records show: mean heart frequency 63.3 bpm (SD 9.79), mean PR length 0.16 (SD 0.57), mean QRS 0.1 (SD 0.04), and mean QT 0.40 (SD 0.04). Most frequent electric alteration sighted was anterior branch block and right bundle branch block. Echocardiographic measurement of the FE displays a mean of 62.2% (SD 6.3) and the left ventricular volume measured in the telediastolic phase means 46.6 (SD 4.7). Patients with diastolic dysfunction, lower ejection fraction, bigger biventricular end-diastolic or end-systolic dimensions had statistically significantly worse cardiac outcome measured by kuschnir classification.
Conclusions Chagas cardiomyopathy affects almost 23% of our study population. Echocardiographic characteristics, like ejection fraction, diastolic dysfunction and ventricular dimensions were associated with heart affectation. These parameters could predict silent cardiac involvement before than the classical kuschnir classification.

P.1.4.9.016 (B)

The pentamidine-miltefosine combination as an alternative to liposomal amphotericin B for treating *Leishmania infantum* visceral leishmaniasis in the immunodeficient host

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Introduction Liposomal amphotericin B (LAmB) may fail to heal Leishmania infantum (Li) visceral leishmaniasis (VL) in the immunodeficient host. In such cases, alternatives are needed.
Material and Methods Clinical cases reporting.
Results A man was coinfected with HIV and C hepatitis and his HIV viral load was undetectable since 2005. A diagnosis of VL was established in 2005. His CD4 count was 162/μl. LAmB was administered from 2005 until 2009, when it was discontinued because of a deafness (total dose: 14.6 g). A treatment with intravenous (IV) pentamidine (total dose: 40 mg/kg) was administered, followed by 7 months of oral miltefosine. Clinical and parasitological outcome were favourable and no relapse was observed 3 years later. Mrs B had a visceral leishmaniasis diagnosed in july 2010 (CD4 = 62/μl). She had an HIV infection and her viral load was undetectable since 2008. VL was unsuccessfully treated with LAmB (total dose: 15.6 g). Eventually, a treatment with pentamidine (total dose: 40 mg/kg) was administered, followed by a 3 months course of miltefosine and a maintenance therapy with pentamidine. Clinical and parasitological outcomes were favourable (total blood PCR was negative). Maintenance therapy was suspended provided that the CD4+ cell count was maintained at >200 cells/îl for more than 6 months along with negative total blood PCR assessments. A relapse of leishmaniasis was observed 5 months later.
Conclusions When LAmB fails or is contraindicated, IV pentamidine followed with oral miltefosine may be an efficient alternative.

P.1.4.9.017 (B)

Leishmaniasis in Suriname – an integrated programme

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According to text books, leishmaniasis in Suriname is cutaneous leishmaniasis caused by *Leishmania guyanensis*, and can be adequately treated with pentamidine. The sand fly vectors and possible reservoir are not well known. A Suriname – Netherlands research consortium is currently studying in an integrated manner several aspects of the disease in order to gain more in depth knowledge on the disease in the country and to contribute to a control programme for leishmaniasis in Suriname, which is currently not available. The research programme comprises three projects: (i) Biological aspects of the parasite and vector; (ii) Clinical aspects of disease and (iii) Medical Anthropology. Biological research on the epidemiology of the parasite has revealed that next to *L. guyanensis*, at least two other species are present in Suriname, including the muco-cutaneous leishmaniasis(MCL) causing *L. braziliensis*. Medical doctors (including from EU) treating cases from the interior of Suriname for CL must be aware that next to CL, also MCL could be contracted This finding has therapeutic implications since the first line recommended treatment for *L. braziliensis* infections is not standard in Suriname. At least three, for Suriname new, sand fly species have been identified and molecular analysis revealed that these sand flies can be infected. Reservoir studies are on-going. Clinical research has demonstrated that pentamidine may not be efficacious for all cases of CL found and alternative treatment regimens are being explored. Effect of drugs can be well monitored over time by using a recently developed RT PCR that may even be able to predict treatment outcomes. Medical anthropology has revealed that stigmatization of infected individuals may not be a major problem in the social acceptability of the disease. Many non-conventional methods, including use of dangerous chemicals, are practised, in particular in the interior of Suriname, to treat the disease.

P.1.4.9.018 (B)

Emerging risk for leishmaniosis in north-eastern Italy: preliminary results of a 12-years entomological survey

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Introduction Since the 1990s, new foci of Canine Leishmaniosis (CanL) have being detected in northern Italy, previously regarded as non-endemic. The study describes the results of the 12-years entomological survey in north-eastern Italy, aimed to better understand the ecology of the vector and the epidemiology of the disease.
Materials and Methods Sandfly trapping was conducted from 2001 to 2012 in 176 sites, using sticky traps for two consecutive nights, CDC light traps and CO_2 traps for one night. A total of 114 sites were monitored by sticky, 54 by CDC and 66 using CO_2 traps. At each location, altitude, structural characteristics of site, level of urbanization and species of domestic bait animals were registered. Associations with sandflies presence/absence and density values were explored using the Pearson chi-square test and Kruskal–Wallis/Mann–Whitney *U*-tests, respectively.
Results Sandflies were observed at 95 of 176 sites monitored (54%). Overall, 6145 sandflies were collected and identified as *Phlebotomus perniciosus* (n = 3798, 60.9%), *P. neglectus* (n = 665, 10.9%), *P. papatasi* (n = 45, 0.7%), *P. mascittii* (n = 29, 0.2%) and Sergentomya minuta (n = 1065, 17.3%); 543 sandflies could not be identified. Only altitude was found to be significantly associated with sandfly presence and densities: the intermediate altitudinal sites (100–300 m.a.s.l.) showed a percentage of positive (73.9%) and an average density (16.3 sandflies/m^2) higher than sites at lower (<100 m) and higher (>300 m) altitude.
Conclusions Our study showed the presence of *P. perniciosus* and *P. neglectus*, both proven vectors of *Leishmania infantum*, in selected areas of north-eastern Italy with density values comparable to endemic areas of southern and central Italy, and suggested that hilly areas up to the altitude of 300 m represent the optimal environment for sandflies. Currently, the ecological requirements of the sandfly vectors are being explored in more detail to facilitate prediction of leishmaniosis risk areas in the region to be targeted for control.

P.1.4.9.019 (B)

Molecular diagnosis of leishmaniasis in Sweden

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Leishmaniasis is a vector-borne disease caused by parasitic protozoa. The disease is endemic in 88 countries and an estimated 350 million people are at risk of infection. About 21 Leishmania species cause several clinical syndromes including visceral, cutaneous and mucosal leishmaniasis. Treatment is complicated by the complexities of this disease and the lack of data from clinical trials. The parasitology unit at Smittskyddsinstitutet (SMI) uses an integrated approach to the diagnosis of leishmaniasis. Methods include antibody detection, microscopy, *in vitro* culture and molecular diagnosis. Species differentiation is performed by restriction fragment length polymorphism (RFLP) since 2010 and prior to this monoclonal antibodies were used. A retrospective, descriptive study was performed to describe the specimens submitted for the molecular diagnosis of Leishmania in Sweden. The analysis included patient demographics, country of infection and Leishmania species. From January 2010 to April 2013, 147 specimens from 129 patients were received at SMI for molecular diagnosis. Of these specimens, 28% (41/147) were PCR-positive. Species differentiation was performed on 38 specimens from 33 patients; with the most common being *L. major* followed by *L. donovani* complex and *L. tropica*. Travel histories included the Middle East, Africa, Central and South America, Asia and southern Europe; with the majority of patients infected in Afghanistan. The ratio of male to female patients was 1.6:1 and the age range was 1–72 years. Twenty-six patients had cutaneous manifestations, six patients had symptoms of visceral leishmaniasis and one patient mucosal leishmaniasis. Travel, immigration and the influx of refugees from areas of conflict bring imported cases of leishmania to Sweden every year. An integrated approach to the diagnosis and follow up of patients with leishmaniasis is essential.

P.1.4.9.020 (B)

An outbreak investigation of Kala-azar in tribal community of eastern Nepal

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INTRODUCTION Kala-azar (KA) elimination programme is ongoing since 2005. Recently, there was an outbreak in Majhare, village in Morang district which borders Bihar, India. Our objective was to investigate the outbreak and propose interventions for prevention.
METHODOLOGY Medical records at the District Public Health Office, Morang district was retrieved. A household survey was conducted in December 2013 covering all 274 households (1152 individuals) which included. socio-demographic information, signs & symptoms suggestive of KA and health seeking behavior. All the febrile cases were screened with rK39 rapid strips test to detect active cases of KA. We also collected blood samples from all individuals of aged >2 years to detect Leishmania infection.
RESULTS A total of 21 new KA cases were identified with onset of symptoms ranging from 2 weeks to 6 months prior to the active case detection. Two deaths had occurred before treatment could be started. KA incidence increased from 52 per 10 000 population in 2011 to 182 per 10 000 population in 2012 suggesting an outbreak. A total of 22 households were affected with KA and there were nine households with more than one cases. Past records and household survey identified the first case in this foci occurring in 2009 in a migrant laborer who had returned from India. The villagers had poor knowledge about KA. None of the houses were sprayed with insecticides in the past due to non cooperation of the villagers. On investigation for Leishmania infection by serology is ongoing.
CONCLUSION Migration, lack of vector control activities, existing housing conditions and living style all are conducive for continuation of disease transmission. Health education campaign for villagers, routine vector control activities and the surveillance system should strengthened (including initiation of active case-finding measures) for prevention of such outbreaks in the future.

P.1.4.9.021 (B)

Automated indirect immunofluorescence for routine laboratory diagnosis of leishmaniasis

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INTRODUCTION *Leishmania infantum* is a zoonotic parasite that is endemic in northern Tunisia. Infected dogs play an important role in the transmission of the parasite to humans. Thus, information regarding prevalence and geographic distribution of canine infections is essential for developing and monitoring strategic control measures. Analyses of antibodies by indirect immuno-fluorescence (IIF) are the most commonly used test in canine leishmaniasis survey. Automation of antibody IIF reading including pattern recognition may be least time consuming and meet the demand for cost-effective assessment of large numbers of samples. In This work, we developed a new automated processing for IIF slides and interpretation of immunofluorescence images of canine antileishmanial antibodies.
MATERIAL AND METHODS IIF assay tests were performed to detect anti-Leishmania antibodies in a serum of a naturally infected dog using an antigen prepared from a reference strain of the *L. infantum* MON-1 zymodeme and an anti-DOG IgG F(c) (RABBIT) antibody fluorescein conjugated. A dilution of 1:800 was considered the cutoff point. For each dilution an IIF image sample was collected with an optical fluorescence microscope. To obtain accurate cell boundaries in our images, we applied a cell segmentation using Hessian- Based method detection. Then, we calculated features that serve as input to a random forest classification method to automatically decide whether image contains the amastigote parasite form.
RESULTS Compared to other methods for biomedial image segmentation, the proposed method produces better results in low-resolution images. Preliminary results show that, in a noisy image, the detection of the amastigote parasite form by the proposed method needs to be improved.
CONCLUSION Owing to its performance characteristics, automated interpretation can overcome limitations of classical IIF for antileishmanial antibodies detection. Although it seems a promising technique, it needs further evaluation on larger serum samples for application in routine laboratory diagnosis of leishmania.

P.1.4.11.022 (B)

A rationale for the low prevalence of visceral leishmaniasis in Sri Lanka: immunization with a naturally-attenuated cutaneous *L. donovani* parasite protects against visceral leishmaniasis in mice

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Leishmaniasis is a neglected tropical disease caused by Leishmania protozoa. It is associated with a range of clinical manifestations, from self-resolving cutaneous lesions to severe visceral leishmaniasis. The infecting species is an important determinant of disease phenotype; *Leishmania donovani* is the major causative agent of visceral leishmaniasis in Southeast Asia. However, in Sri Lanka, there have been over 2000 cases of cutaneous leishmaniasis caused by *L. donovani* in the past 10 years while only four cases of visceral leishmaniasis have been reported. To explain the scarcity of visceral leishmaniasis in Sri Lanka, we hypothesized that prior infection with the more prevalent cutaneous parasite protects against visceral disease. Two clinical *L. donovani* isolates were obtained, one from a cutaneous lesion (SL-CL) and one from a visceral leishmaniasis patient (SL-VL). BALB/c mice were immunized subcutaneously with SL-CL and challenged intravenously with SL-VL. Significant protection following short-term immunization was observed in the liver for mice immunized with SL-CL doses of 10^4 and higher. Liver protection was durable (maintained at 3 months post-immunization) and did not require persistence of the immunizing parasite dose. Protection for the short-term challenge was associated with a mixed Th1/Th2 response prior to challenge, with higher IFNg and IL4 production in protected groups, and no antigen-specific IL10 production. The post-challenge immune response was similar for long-term and short-term challenge, with comparable IFNg production in all groups and elevated IL4 and IL10 production in protected groups. Given that most individuals exposed to Leishmania parasites do not develop symptomatic disease, exposure to the cutaneous *L. donovani* parasite in Sri Lanka may shift the balance towards enhanced asymptomatic cases, thereby leading to the observed low prevalence of visceral leishmaniasis. Finally, these results provide a framework for re-evaluating live immunization against visceral leishmaniasis and as such could guide leishmaniasis vaccine development efforts.

P.1.4.9.023 (B)

Evaluation of synthetic peptides from the K39-sequence for diagnostics of visceral leishmaniasis

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Routine ELISAs for diagnosis of viserceral leishmaniasis currently rely on crude antigen preparations from parasites or on recombinant proteins as capture antigens, which are prone to batch to batch variation and to nonspecific cross reactivity. In contrast, synthetic peptides can be synthesized in large amounts, high purity and more reproducible quality. In this study we evaluated different synthetic peptides derived from the kinesin-like protein K39 sequence as coating antigens for serological diagnosis of visceral leishmaniasis. Kinesin-like protein K39 is expressed in different variants by the donovani complex comprising *Leishmania donovani*, *L. chagasi* and *L. infantum*. The C-terminal region of the protein consists of a multi-repeat unit. Several sequences from the multi-repeat region were selected for synthesis, resulting in eight biotinylated peptides of 21–39 amino acids in length. Peptides were coated on streptavidin coated microtiter plates and tested in ELSIA with sera from patients with visceral or cutaneous leishmaniasis, sera from other protozoan or helminthic infections, as well as negative sera from healthy blood donors. The sequence which showed the best sensitivity and specificity was the basis for creation of an artificial peptide with three identical short repeats. This peptide was coated as streptavidin-biotin complex on standard Immulon 2HB microtiter plates and was tested under routine ELISA conditions to determine sensitivity and specificity. Results show high reactivity with visceral leishmaniasis sera and reduced reactivity with cutaneous leishmaniasis sera, which is also described with whole recombinant K39 protein. We observed frequent cross-reactivity with sera from malaria patients, while less cross-reactivity was detected with sera from Chagas' and sleeping sickness patients. Our findings suggest that synthetic peptides are suitable antigens for serological diagnosis of parasitic infections, deliver more reproducible results and are generally easier to access.

P.1.4.9.024 (B)

Genetic homology of *Leishmania major* strains isolated in Tunisia from two rodents spp, revealed by MLMT

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Introduction Zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania* (L.) major parasites represents a major health problem with a large spectre of clinical manifestations. *Psammomys* (P.) *obesus* and Meriones (M.) shawi represent the most important host reservoirs of these parasites in Tunisia. We already reported that infection prevalence is different between these two rodents. We aimed in this work to evaluate the impact of parasite genetic background on this difference.
Material and Methods Using the multilocus microsatellites typing (MLMT), we analyzed the genetic diversity among strains isolated from (i) *P. obesus* ($n = 31$), (ii) *M. shawi* ($n = 8$) and (iii) Mustela nivalis ($n = 1$), captured in Sidi Bouzid, an endemic region for ZCL located in the Center of Tunisia. Ten highly polymorphic microsatellites markers were used to study the genetic structure and micro-heterogeneity of these strains.
Results All markers showed no polymorphism among the analyzed strains regardless of their host-reservoir origins.
Conclusion Absence of genetic variability between strains isolated from different reservoirs can be explained by similarity of geographic and ecological parameters within the study area.

P.1.4.9.025 (B)

Risk factors of childhood Leishmaniasis lesions healing time: a multivariate marginal Cox regression analysis

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Introduction Zoonotic Cutaneous Leishmaniasis (ZCL) is a seasonal and endemic disease in central and southern Tunisia, where it is caused by *Leishmania* (L.) *major*. Transmission of *L. major* occurs during the summer and active lesions in humans tend to emerge during autumn and winter. It affects both adults and children involving often only the skin, and may be characterized by one to dozens of lesions. Most skin lesions heal spontaneously. However, some lesions may persist for long periods or leave scars. This study attempts to elucidate the risk factors associated with ZCL lesion healing time in childhood.
Material and Methods A prospective study was conducted in primary schools from central and southern Tunisia. Eighty three children with 215 ZCL lesions were randomly selected and followed closely for 2 years (April 2001–April 2003) to assess the natural evolution of their skin lesions. The Kaplan-Meier method was used for the time-to-healing analysis. Univariate and multivariate marginal Cox proportional hazard models were applied to determine risk factors for lesion healing time.
Results Survival analysis suggested that median time to heal was 18 weeks. All lesions healed within 8 months. Multivariate analysis indicated that only month of lesion onset had significant effect on healing time. Lesions which emerge early healed faster than those which appeared later, with a significant increasing trend in the adjusted odds ratio of healing time over the 4 months of lesion onset [from September (reference month) to December: 2.1 (Confident Interval [CI] 95%: 1.3, 3.5); 3.2 (CI 95%: 1.7, 6.0); 8.6 (CI 95%: 4.7, 15.6); $P < 0.001$].
Conclusion Large prospective studies of natural history of ZCL lesions are needed to further characterize factors that might influence the healing process dynamics.

P.1.4.9.026 (B)

Role of *Meriones shawi* in the spread of zoonotic cutaneous Leishmaniasis in Central Tunisia using telemetry and mathematical modeling

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Introduction Zoonotic cutaneous leishmaniasis (ZCL) caused by *Leishmania major* is endemic in central and southern Tunisia. *Phlebotomus papatazi* (sandfly) is the proven vector of and rodents *Psammomys obesus* and Meriones spp. serve as animal reservoir hosts. In recent years, several new foci have been reported, indicating the potential spread of the disease in Tunisia. Key factors driving spatio-temporal dynamics of the

disease are presently unknown. These might include dynamics of rodent populations, dispersal of vectors, and natural and man-made environnemental changes. The aim of this work is to investigate the contribution of *Meriones* (M.) shawi in the geographic spread of ZCL in central Tunisia.
MATERIAL AND METHODS An ecological study was undertaken between March and December 2012 in the region of Sidi Bouzid to quantify the spatial dynamic of thirty *M. shawi* by telemetry. Each animal was attached with a radio collar around the neck and monitored for 6 months. We estimated the size of the home range using the minimum convex polygon (MCP) method. A spatial mathematic model based on Fickian Diffusion was developed to describe the *M. shawi* spatial-temporal dynamics.
RESULTS The average distance traveled by rodents during the study period stood at around 0.734 km. The mean home range size with MPC method was 0.1507 km². Using data from previous study, the estimated percapita rate of ZCL infection among *M. shawi* sample was 3.1 Indivdual-1 Year-1 and the diffusion coefficient was 0.6024 km²Year-1. Analysis of different simulation scenarios revealed the high mobility of *M. shawi* which accelerates ZCL infection among rodents.
CONCLUSION To our knowledge, this is the first time that the dynamics of *M. shawi* was assessed in Tunisia using a mathematical approach. It contributes to further incriminate *M. shawi* in the spread of ZCL among humans.

P.1.4.9.027 (B)
Leishmaniasis in Croatia

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Both cutaneous (CL) and visceral leishmaniasis (VL) are endemic in Asia, East Africa, South America and the Mediterranean region. Early diagnosis and treatment of VL is important in order to prevent fatal outcomes. The aim of the study was to evaluate the data on CL and VL in patients treated in Croatia in the period from 1999 to 2012. We conducted a retrospective survey on patients with VL; all infectious disease departments in Croatia were asked to fill out a predefined form. Twenty-one patients with CL were reported to the National Institute of Public Health in the period 1999–2012. Forty patients were treated for visceral leishmaniasis (18 were patients <18 years old). The median time from the onset of symptoms to diagnosis was 22 days. In 30 patients the infection was acquired in Southern Croatia where they lived, while nine patients, out of 10 who lived outside of the VL endemic areas, recalled traveling to VL endemic countries outside of Croatia and in Croatia as well. There were three cases of HIV and leishmania co-infection; one patient had a chronic leishmania infection with four clinical relapses. VL was diagnosed based on bone marrow aspirate cytology, serology and PCR in 37, 31 and 6 patients, respectively. Thirty-three patients were treated with sodium stibogluconate, two patients with amphotericin B colloidal dispersion, two patients with liposomal amphotericin B, and three patients with conventional amphotericin B. Two patients received combination treatment including allopurinol and fluconazole. Side effects were reported in 10 patients (25%), mainly pancreatitis due to sodium stibogluconate treatment. Thirty-four patients were cured. Three patients died. VL is still a sporadic disease in Croatia. The current recommendations on VL treatment (liposomal amphotericin B as a first line treatment) should be implemented in our clinical practice.

1.4.10 Soil-transmitted helmintiasis

P.1.4.10.001 (A)
Chau Cuica: evaluating the impact of a government-initiated school-based deworming program in Loreto, Peru

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INTRODUCTION School-based deworming programs are recommended by the World Health Organization to treat soil-transmitted helminth (STH) infections in endemic countries. In Peru, a national deworming program does not exist; however, in July 2012, the regional government of Loreto implemented its first large-scale school-based deworming program, following a donation of 1 386 000 single-dose mebendazole tablets. Their distribution plan included delivering mebendazole tablets to all schoolchildren in the region every four months. The objective of this study was to monitor the health impact of this new deworming program.
METHODS A pre/post research study design was used in 16 sentinel schools, located in all seven provinces of Loreto. Baseline assessment took place from July 3 to 16, 2012. All schoolchildren in Loreto were dewormed on July 17, 2012. The first monitoring assessment took place in the two weeks immediately preceding the second deworming cycle (November 5–15, 2012). Baseline and monitoring assessments were identical. A total of at least 50 Grade 5 schoolchildren from each sentinel school were recruited into the study at each assessment. Age, sex, height and weight were recorded; hemoglobin levels were measured; and stool specimens were analysed for STH prevalence and intensity.
RESULTS A total of 815 and 835 children were recruited into the study at baseline and at the first monitoring assessment, respectively. Ascaris prevalence decreased from 65.2% at baseline to 43.1% (aOR = 0.35, 95% CI: 0.32, 0.48, adjusted for age and sex). The egg reduction rate (ERR) for Ascaris was 86.2%. Similarly Trichuris prevalence decreased from 64.8% to 40.4% (aOR = 0.37, 95% CI: 0.30, 0.45) with an ERR of 62.1%.
CONCLUSIONS STH infection status was significantly improved following one cycle of deworming. Further improvements in STH infection status and subsequently in hemoglobin levels and growth are expected in future monitoring assessments of this new program.

P.1.4.10.002 (A)
Prevalence of Strongyloidiasis in Latin America: a systematic review of literature

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BRIEF INTRODUCTION Strongyloides stercoralis is rarely recognized as a major public health issue, probably in relation to the underestimation of its burden. In fact, estimates of the worldwide prevalence of strongyloidiasis are mostly based upon surveys conducted with diagnostic methods which have demonstrated low sensitivity. The aim of this systematic review was to collect available

information about the prevalence of strongyloidiasis in an area of presumable high endemicity: Latin America.
MATERIAL AND METHODS We conducted an electronic search in Pubmed, collecting studies published from the 1st January, 1991 to the 1st January, 2011. Search was limited to 'humans' and to the following languages: English, Spanish, Italian, French. Moreover, grey literature was searched.
RESULTS Pubmed search permitted to identify 1071 papers, of which 79 were included in the analysis. Grey literature search permitted to add other nine papers, therefore the total number of included papers was 88. We found data about the following countries: Argentina (10 papers), Bolivia (2), Brazil (31), Chile (5), Colombia (3), Costa Rica (3), Ecuador (7), Guatemala (1), Guyana (1), Honduras (3), Mexico (2), Nicaragua (1), Peru (12), Puerto Rico (2), Venezuela (5). Studies were heterogeneous in relation to different populations screened and diagnostic methods used. The large majority of studies relied on stool microscopy, often without any of the enrichment techniques which can enhance the detection of Strongyloides larvae. Serology was done only in six cases. The following countries presented areas of very high prevalence (>20%): Argentina, Ecuador, Venezuela, Peru (in particular the Amazon basin), and Brazil (id).
DISCUSSION Globally, for most of the included countries it is not possible to define a reliable level of prevalence of strongyloidiasis because of scarceness and or/inadequacy of studies. Strongyloides requires specific diagnostic methods for its detection, therefore surveys should be specifically designed, in order to avoid underestimation.

P.1.4.10.003 (A)

Ascaris lumbricoides in Rwanda: associated factors and effectiveness of school-based preventive chemotherapy

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Ascaris lumbricoides is the predominant soil-transmitted helminth (STH) in the Southern Province of Rwanda. School-based deworming is applied twice annually but its actual effectiveness is unknown. In the area of Butare, we assessed factors associated with *A. lumbricoides*-infection and the effectiveness of preventive chemotherapy. Children were recruited at one urban and one rural school. Stool samples were collected before mebendazole treatment (500 mg single dose), and after 2 and 12 weeks, respectively. STHs were diagnosed by microscopy of Kato-Katz slides and by PCR assays for *A. lumbricoides*. Socio-economic status and proximate risk factors for STH infection were assessed by structured questionnaires. Pre-treatment stool samples were collected from 333 urban and 329 rural school children (median age, 10 years; range, 5–17). By microscopy, 17.2% of the children harboured STHs (*A. lumbricoides*, 16.0%; *Trichuris trichiura*, 1.8%; hookworm, 0.3%), three quarters of infections being of light intensity. Adding PCR results, the pre-treatment prevalence of *A. lumbricoides* was 24.0% which declined to 1.8% 2 weeks after deworming and increased to 6.8% after further 10 weeks. Of the initially *A. lumbricoides* infected children, 5.9% and 18.0% harboured the parasite 2 and 12 weeks, respectively, following treatment. At recruitment, *A. lumbricoides* was more common in rural (37%) than in urban (11%) school children. Further associated factors included low socio-economic status, water supply, and garbage disposal, among others. Similar factors were linked to re-infection. *A. lumbricoides* affects approximately one in four school children in the Butare area of southern Rwanda and is associated with rural residence, low socio-economic status, unsafe water, and behavioural factors. Preventive chemotherapy with mebendazole in this area is effective despite some degree of parasite persistence. Health education and environmental improvement as well as monitoring potential drug resistance are needed to further reduce the burden of *A. lumbricoides* and other STHs in this area.

1.4.12 Global hepatitis

P.1.4.12.001 (B)

Chronic hepatitis B virus infection in pregnant Chinese women living in Barcelona (Spain)

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INTRODUCTION The World Health Organization (WHO) estimates between 350 and 400 million people in the world infected with Hepatitis B Virus (HBV), one third of these are found in China, where around 9% of the population is a chronic carrier of this virus and a half of those obtain those infection through vertical transmission. This study aims to evaluate the prevalence and characteristics of chronic Hepatitis B infection in Chinese pregnant women living in Barcelona South Metropolitan area.
MATERIAL AND METHODS Cross-sectional study between July and December of 2012 evaluating serological markers of HBV in Chinese pregnant women who received prenatal care in the public health system (ASSIR) of Catalan Institute of Health. All tests were performed at the Laboratori Clinic of L'Hospitalet (Barcelona). Hepatitis B surface antigen (HBsAg), Hepatitis B core antibody (anti-HBc), Hepatitis B e antigen (HBeAg) and Hepatitis e antibody (anti-HBe) were analyzed by Chemiluminescence immunoassays(Vitros® Johnson & Johnson) and HVB DNA viral load was quantified by Abbott RealTime HBV DNA®. The immune tolerant phase was defined by HBeAg seropositivity, viral load >107 UI/ml and normal alanine aminotransferase (ALT) levels (<40 UI/l).
RESULTS Of the 62 Chinese pregnant women, 4 (6.5%) of whom were HBV infection and two of them were in Inmune Tolerant Phase. The results of serological patterns are as follows in the table below.
CONCLUSIONS The results of our study show an intermediate presence of chronic VHB infection and elevated presence of markers of VHB past infection .Of significant note is that half of VHB infections are during the immune tolerant phase, characterized by high replicate activity. Pregnant women with high viral loads, and HBeAg seropositivity can result in vertical transmission of infection despite using prophylactic measures for newborns (i.e. vaccination and specific immunoglobuline).

Hepatitis B serological patterns	Subjects (%)
Chronic infection:HBsAg +	4 (6.5)
Inactive chronic carrier HBe Ag (−) ALT normal	2 (3.2)
Inmune tolerant phase HBe Ag(+)ALT normal	2 (3.2)
Past infection:anti-HBc (+) HBsAg (−)	26 (41.9)
No exposure: anti-HBc (−)	32 (51.6)
Total	62

1.4.13 Other NTDs

P.1.4.13.001 (B)

Effective treatment of Tungiasis by topical application of Dimeticone

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Tungiasis (sand flea disease) is a neglected tropical disease associated with debilitating acute and mutilating chronic morbidity. Hitherto, there is no effective drug treatment. In the endemic areas patients attempt to remove embedded sand fleas with inappropriate sharp instruments, a hazardous procedure by itself. The efficacy of a topical application of a mixture of two dimeticones of low viscosity (NYDA®) on embedded sand fleas was assessed in 47 school children in rural Kenya. It was compared to bathing the feet in 0.05% KMnO4 (the traditional treatment in Kenya). The viability of the parasites was assessed through a handheld digital video microscope and the degree of inflammation was determined by an inflammation score. The lesions were observed daily during 7 days. In the dimeticone group, 78% (95% CI 67–86%) of the embedded sand fleas had lost all viability signs after 7 days. In the KMnO4 group viability signs disappeared in 39% (95% CI 28–52%; $P \leq 0.001$). The inflammation score decreased from 6 to 4.75 points in the dimeticone group ($P < 0.001$), but remained unchanged in the KMnO4 group (4.5 vs. 5 points). Based on the established physical mode of action of dimeticones on other ectoparasites, it is assumed that NYDA® rapidly crept into the respiratory tract of the embedded sand fleas and caused death through hypoxia. In view of the efficacy and safety of dimeticones, the hazardous extraction of embedded sand fleas with sharp instruments is no longer warrantable.

1.5 Malaria

1.5.1 Malaria immunology

P.1.5.1.001 (A)

Toll-like receptor polymorphisms affect monocyte responses towards *Plasmodium falciparum*-infected erythrocytes

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Single nucleotide polymorphisms (SNPs) of Toll-like receptors (TLR) have been linked to susceptibility to human malaria in epidemiological studies. However, their functional roles still remain unclear. *Plasmodium falciparum* is reportedly recognized by TLR2 (TLR2/1, TLR2/6), among others. We investigated the impact of common functional TLR1 and TLR6 SNPs on the cytokine response towards *P. falciparum*-infected erythrocytes (iRBCs). Malaria-naive, healthy blood donors were screened for TLR SNPs (TLR-1, TLR-6, TLR-2 etc.). Peripheral blood mononuclear cells (PBMCs) and monocytes were isolated from blood samples and co-cultivated with iRBCs. Supernatant cytokine concentrations were determined by ELISA. IL-1ß and IL-6 responses of human monocytes towards iRBC showed heterogeneity between monocyte donors and depended on the ratio of effector cell to iRBC. Monocytes from malaria-naïve donors with TLR-variants associated with increased risks of malaria (TLR1 I602S; TLR6 S249P) showed reduced release of IL-1ß and IL-6 in response to iRBC. Homozygous TLR-6 249P revealed a pronounced effect of reduced iRBC-stimulated IL-6 production. Cytokine responses were lowest for monocytes from donors with both SNPs. In a West African population, both SNPs were found to be remarkably scarce. TLR SNPs associated with increased malaria risk correspond to a reduced monocyte production of pro-inflammatory cytokines in response to exposure with *P. falciparum*. This suggests a functional role of the SNPs and of the respective TLRs in susceptibility to malaria. Curbed initial innate responses to malaria parasites may favor the development of clinical disease, and the disadvantage of respective genetic disposition may explain negative selection in endemic regions.

P.1.5.1.002 (A)

Long-lived *Plasmodium falciparum*-specific memory B cells in naturally exposed Swedish travellers

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INTRODUCTION Antibodies (Ab) are critical for immunity to malaria. However, *Plasmodium falciparum*-specific Ab decline rapidly in absence of re-infection, suggesting impaired immunological memory. This study determines whether residents of Sweden that were treated for malaria following international travel maintained long-lasting malaria-specific Ab and memory B cells (MBC).
MATERIAL AND METHODS We compared levels of malaria-specific Ab and MBC between 47 travelers who had been admitted with malaria at the Karolinska University Hospital between 1 and 16 years previously, eight malaria-naïve adult Swedes without histories of travel, and 14 malaria-immune Kenyan-adults.
RESULTS *P. falciparum*-lysate-specific Ab levels, were above naïve-control levels in 30% of the travelers, whereas AMA-1, MSP-142 and MSP-3-specific Ab levels were similar. In contrast, 78% of travelers had IgG-MBC specific for at least one malaria-antigen (59%, 45% and 28% for AMA1, MSP1 and MSP3, respectively) suggesting that malaria-specific MBC are maintained for longer than the cognate serum Ab in the absence of re-exposure to parasites. Five travelers had maintained malaria-antigen specific MBC responses for up to 16 years since the diagnosis of the index episode (and had not traveled to malaria endemic regions in the intervening time).
CONCLUSION *P. falciparum* can induce long-lasting MBC, maintained for up to 16 years (or more), without re-exposure.

1.5.2 Malaria and iron deficiency

P.1.5.2.001 (B)

Total body iron and IPTp calendar are associated with *Plasmodium falciparum* parasitemia during the first year of life in Benin

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INTRODUCTION In Benin malaria is the leading cause of infant mortality, responsible for 23% of deaths among children under

5 years. In this context co-morbidities like anaemia (present in >80% of the children) contribute significantly to worsen the disease. In parallel, IPTp has modified the epidemiology of the disease improving LBW statistics and clearing placenta parasites. Hence it is necessary to clarify the risk factors for infant parasitemia.
Methods Four hundred pregnant women and their children were followed during pregnancy and the first year of life. At inclusion socio-demographic characteristics and gyneco-obstetric history were documented. Anthropometric measures, haemoglobin, blood smear, blood film, seric ferritin, CRP, folic acid, B12 vitamin, haemoglobin electrophoresis were realized at both IPTp consultations and at delivery. Parasitemia, anthropometric measures, haemoglobin, seric ferritin, CRP were measured at 6, 9, and 12 months. Further exams were realized during emergency consultations. Random coefficient models were used to assess the relationship between the different parasitemia measures in infants and other variables.
Results Maternal age at both IPTp doses, weight of the infant, mother parasitemia at delivery, number of emergency consultations and total body iron are correlated with infant parasitemia. Placental malaria is not correlated with infant parasitemia when adjusting for mother parasitemia at delivery.
Conclusion IPTp has not only an effect on LBW but also on infant parasitemia. Total body iron is also correlated with infant parasitemia. Hence, these considerations should be taken into account for policy recommendations regarding IPTp calendar and iron infant supplementation.
Key words Malaria, IPTp, TBI, Parasitemia

P.1.5.2.002 (B)

Correlation of malaria infection and anemia in children under 5 years of age without fever in the health zone of Mont Ngafulal a hyper endemic area for malaria in Kinshasa, democratic Republic of Congo

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In 2010, there was an estimated 216 million episodes of malaria, of which approximately 81%, or 174 million cases occurred in the African region. Moreover, the estimated number of deaths due to malaria was 655 000, of which 91% still took place in Africa. Yet, results of effective intervention studies suggest that the true number could even be higher because of indirect effects of the disease on other health related issues like anemia. Therefore the aim of the study was to search for the correlation between the presences of malaria infection due to *Plasmodium falciparum* and anemia in a highly endemic area in children under 5 years of age. A cross sectional household based survey was conducted in the Health Zone of Mont Ngafula1, Kinshasa, during the dry season from April to August 2012. Informed consent, blood smears and the hemoglobin results were obtained from 700 children. The proportion of anemia in the global population was 51.1% (CI 95%: 47.4–54.9). Among the children with a positive blood smear, 76% presented anemia compared to 42% in the group with a negative blood smear ($P < 0.000$). The presence of anemia was also assessed within three different classes of parasitic density (<1000 p/ml, 1000–2000 p/ml, >2000 p/ml) but no significant statistical differences could be found. These results tend to show a correlation between the presence of malaria infection and the anemia status of children in a hyper endemic area for malaria.

1.5.3 Experimental malaria

P.1.5.3.001 (A)

Protective effect of Myeloperoxidase-deficiency in *Plasmodium yoelii* infection

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Objective Myeloperoxidase (MPO), a leukocyte-derived enzyme catalyzing the generation of reactive oxygen species (ROS) and the oxidation of nitric oxide (NO) is a key player in inflammation and has long been known for its bactericidal properties. Its role in parasite defense is yet unclear. Oxidative burst and NO are believed to be directly involved in parasite killing in malaria infections. We analysed the role of MPO in the immune defense in murine malaria models.
Material and Methods C57BL/6J and MPOtm1lus mice were infected with *P. yoelii* 17NL with and without prior antibody-mediated neutrophil depletion. Parasitaemia was assessed from tail blood. NO, inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) were determined and correlated with isometric tension studies of aortic rings.
Results *P. yoelii* infected wildtype mice show high MPO plasma levels. However, depletion of neutrophils, the main producers of MPO, before infection or deficiency of MPO did not increase parasite levels in *P. yoelii* infection. In contrast, MPO-deficient mice display a decrease in parasitaemia. NO bioavailability as well as gene expression of iNOS and eNOS was also decreased in MPO-deficient mice.
Conclusion Despite the established role of ROS as well as NO in parasite killing, the depletion of neutrophils or the deficiency of leucocyte-derived myeloperoxidase as the main producer of ROS did not impair the control of *P. yoelii* infection. Instead, MPO-deficiency seems to attenuate parasitaemia in infected mice. We assume that MPO may play a role in erythrocyte adhesion to the endothelium and, hence, the avoidance of parasite clearance by the spleen.

P.1.5.3.003 (A)

Erythrocytic apoptosis in *Plasmodium yoelii* malaria is related to parasite load, but not to immune response or anaemia degree

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Recently, through the study of erythrocytic apoptosis during *Plasmodium yoelii* infection, we observed a rise in the levels of non-parasitized red blood cells (nRBC) apoptosis that could be related with severe malaria anaemia, as premature elimination of nRBC is a relevant mechanism leading to this malaria complication. In the present study, therefore, we attempt to investigate if nRBC apoptosis is associated with peripheral RBC count, para-

site load or immune response. To this end, Balb/c mice were intraperitonially infected with *P. yoelii* 17XL and, then, nRBC apoptosis as well as number of peripheral RBC, parasitaemia and plasmatic levels of cytokines, nitric oxide and anti-RBC antibodies were evaluated at early and late anaemia stages, days 4 and 7 post infection, respectively. Apoptosis of nRBC was increased only at the late stage and it was associated to parasite load, but not to the intensity of the immune response. In spite of increased percents of nRBC apoptosis observed when the anaemia was accentuated, this increase was not related to the reduction of peripheral RBC counts, suggesting that apoptosis is not significantly implicated in anaemia observed in the malaria model studied herein. We conclude that nRBC apoptosis in *P. yoelii* malaria seems to be induced in response to high parasite load and that further studies on malaria models in which acute anaemia develop under low parasite burden are needed to identify the potential pathogenic role of nRBC apoptosis.

1.5.4 Challenges in anti-malarial drug resistance

P.1.5.4.001 (B)

Safety and efficacy of Co-trimoxazole for treatment and prevention of *Plasmodium falciparum* malaria: a systematic review

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INTRODUCTION Cotrimoxazole (CTX) has been used for half a century. It is inexpensive hence the reason for its almost universal availability and wide clinical spectrum of use. In the last decade, CTX was used for prophylaxis of opportunistic infections in HIV infected people. It also had an impact on the malaria risk in this specific group.
MATERIALS AND METHODS We performed a systematic review to explore the efficacy and safety of CTX used for *P. falciparum* malaria treatment and prophylaxis.
RESULT CTX is safe and efficacious against malaria. Up to 75% of the safety concerns relate to skin reactions and this increases in HIV/AIDs patients. In different study areas, in HIV negative individuals, CTX used as malaria treatment cleared 56–97% of the malaria infections, reduced fever and improved anaemia. CTX prophylaxis reduces the incidence of clinical malaria in HIV-1 infected individuals from 46% to 97%. In HIV negative non pregnant participants, CTX prophylaxis had 39.5–99.5% protective efficacy against clinical malaria. The lowest figures were observed in zones of high sulfadoxine-pyrimethamine resistance. There were no data reported on CTX prophylaxis in HIV negative pregnant women.
CONCLUSION CTX is safe and still efficacious for the treatment of *P. falciparum* malaria in non-pregnant adults and children irrespective of HIV status and antifolate resistance profiles. There is need to explore its effect in pregnant women, irrespective of HIV status. CTX prophylaxis in HIV infected individuals protects against malaria and CTX may have a role for malaria prophylaxis in specific HIV negative target groups.

P.1.5.4.002 (B)

The Eurartesim® safety registry

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INTRODUCTION A registry is a study that collects health information from people with a particular illness or taking a particular medication. Participation in the Eurartesim® safety registry will add information about exposure to this drug for the treatment of uncomplicated malaria in real life. It has been approved by the European Medicine Agency (EMA) and the competent authorities as required by local regulations and is active in Belgium, France, Germany, Italy, The Netherlands, Spain and the UK.
MAIN PURPOSE This safety registry is an active, prospective, voluntary surveillance programme to collect as much medical information as possible on people administered with Eurartesim® for the treatment of imported uncomplicated malaria (*Plasmodium falciparum*) in European countries.
MATERIAL AND METHODS It will collect prospective medical and life-style data by means of ad hoc questionnaires covering information on patient's demography and malaria history, co-morbidities, co-medication use, food intake, smoking and alcohol consumptions. These data will be used to investigate the impact of these factors on patient's safety, for example ECG parameters. Personal data will not be disclosed; all medical information will be protected in compliance with the relevant data protection law. Criteria for enrolment: The following patients are eligible for this safety registry:

1 Patients diagnosed with uncomplicated malaria (*Plasmodium falciparum*); diagnosis will have to be clinically and parasitologically confirmed.
2 Patients who agree to participate, by signing the relevant informed consent form.

Participation in another study at the time of this safety registry is an exclusion criterion.

We encourage the physicians to refer all cases of Eurartesim® administration for the treatment of malaria and this poster will be a contribution to the awareness campaign.

P.1.5.4.003 (B)

Analysis of pfcrt and pfmdr1 genes in *Plasmodium falciparum* samples

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Plasmodium falciparum resistance to antimalarials remains an obstacle to malaria control. The pfcrt K76T has been associated to chloroquine resistance, as well as pfmdr1 N86Y and D1246Y, but this association is not well defined. We aimed to analyze these mutations in *P. falciparum* samples and verify its relation with *in vivo* and *in vitro* responses. Samples were collected in Brazil from patients with positive thick smear for *P. falciparum*, enrolled at SUCEN/HCFMUSP and Divisão de Endemias 9° Centro Regional de Saúde de Santarém, from 1984 to 2010. Nested-PCR confirmed *P. falciparum* single infection. SNPs were assessed by PCR-RFLP with restriction enzyme ApoI for pfcrt K76T and pfmdr1 N86Y, and Eco321 for pfmdr1 D1246Y.

Patients were treated according to the guidelines by the time of infection. A sampling was selected for determination of Minimal Inhibitory Concentration (MIC) using *in vitro* tests for chloroquine, quinine and mefloquine. This study was approved by CONEP/Ministry of Health. A total of 130 samples were assessed, 96 from Brazil. Among them, 82.3% contained pfcrt K76T; all Brazilian samples were mutant. Regarding to pfmdr1, 14.6% presented N86Y and 82.3% D1246Y. Recrudescence occurred in 13.1% of patients: one to mefloquine, two to doxiciclin, seven to quinine and seven to artemisinin derivatives. All but one recrudescent presented pfcrt K76T and pfmdr1 D1246Y. All the 22 samples tested for chloroquine *in vitro* presented MIC≥32 pmol/well; two presented MIC = 256 pmol/well for quinine, indicating resistant phenotype. No sample showed resistant phenotype to mefloquine. The pfcrt K76T is still present in *P. falciparum* Brazilian population despite of chloroquine has no longer been used for this species. On the other hand, chloroquine is used for *P. vivax*, which corresponds to 86.9% of infections in Brazil. Therefore, parasites continue under chloroquine pressure. Interestingly, the genotype D1246Y was observed in the same percentage that pfcrt K76T.

P.1.5.4.004 (B)

Mass balance and metabolism of the antimalarial pyronaridine in healthy volunteers

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The antimalarial pyronaridine tetraphosphate has been co-formulated with artesunate in an oral artesmisinin based combination therapy indicated for the treatment of acute uncomplicated malaria in children and adults. An open label, single dose study designed to characterize pyronaridine mass balance and metabolites in humans was conducted with six healthy male volunteers. Each subject was administered an oral dose of 720 mg pyronaridine tetraphosphate with microdose radiolabeled 14C-pyronaridine (800 nCi). Urine and feces were continuously collected through 168 h post-dose, with intermittent 48 h collection periods thereafter through 2064 h post-dose. Drug recovery was computed for analyzed samples and then interpolated for intervening time periods in which collection did not occur. The pharmacokinetics of total radioactivity and of pyronaridine in blood were evaluated. Total radioactivity in urine, feces, and blood samples was determined by accelerator mass spectrometry. Pyronaridine concentrations in blood were determined by LC/MS, and metabolite identification performed using LC/MS/MS. The mean cumulative drug recovery up to 86 days in the urine and feces was 23.7% and 47.8%, respectively, with an average total recovery of 71.5% (range 60.3 â€" 82.2%). Total radioactivity remained detectable in urine and feces collected in the final sampling period, suggesting ongoing elimination. Total radioactivity was slowly eliminated from blood, with a mean half-life of 33.5 days, substantially longer than the mean half-life of 5.03 days for the parent compound. Metabolite characterization allowed for identification of nine primary and four secondary metabolites of pyronaridine, with prominent pathways for metabolite formation including N-dearylation, glucuronidation, and oxidation. This study revealed that pyronaridine and its metabolites are eliminated in humans by both the urinary and fecal routes over an extended period of time, and that multiple, varied pathways characterize pyronaridine metabolism.

P.1.5.4.005 (B)

The analysis of the factor affecting the outbreak of resistant type malaria: application of macro-and micro-epidemiology to the control of the drug-resistant type

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Brief Introduction The spread of drug resistance is making malaria control increasingly difficult. Mathematical models for the transmission dynamics of drug sensitive and resistant strains can be useful tools to help understand the factors that influence the spread of drug resistance, and they can therefore help design rational strategies for the control of drug resistance.
Materials and Methods We present an epidemiological framework to investigate the spread of anti-malarial resistance. Several mathematical models, based on the familiar Macdonald-Ross model of malaria transmission, enable us to examine the processes and parameters that are critical in determining the spread of resistance. We used Mathemacica to analysis.
Results In our simplest model, resistance does not spread if the treated fraction population of infected individuals is less than a threshold value and if drug treatment exceeds this threshold, resistance will not be eventually eradicated in the population. The threshold value is determined by the rates of infection, the infectious periods of resistance and sensitive parasites in untreated and treated hosts; whereas the intensity of transmission has no influence on the threshold value. In more complex models, where hosts can be infected by multiple parasite strains or where difference in treatment methods results in variation in the mode of human-to-human an transmission, resistance is generally not fixed, but rather some level of sensitivity is often maintained in the population.
Conclusion The models developed in this paper are a first step in understanding the epidemiology of anti-malarial resistance and evaluating strategies to reduce the spread of resistance. However, specific recommendations for the management of resistance need to wait until we have more data on the critical parameters underlying the spread of resistance: drug use, different treatment methods resulting in spatial variability of transmission, and parasite migration among areas, perhaps most importantly, and cost of resistance.

P.1.5.4.006 (B)

In vitro susceptibility of *Plasmodium falciparum* to antimalarial drugs 5 years after the change for treatment policy of uncomplicated malaria in Burkina Faso

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Introduction Resistance to commonly used antimalarial drugs represents the major drawback and obstacle for controlling malaria in endemic countries. Burkina Faso has changed in 2005 its

antimalarial drug policy for the treatment of uncomplicated malaria from Chloroquine to Arthemether-Lumefantrine and Amodiaquine + Artesunate. This study, conducted in Bobo Dioulasso, aims to study at comparing the *in vitro* sensitivity of the different ACT components with the results obtained various components of ACTs used in Burkina Faso and current antimalarial drugs after the implementation of effective use of ACT.
MATERIALS AND METHODS The study was conducted from July 2009 to February 2010. Blood samples were collected from patients with a parasitemia between 4000 and 200 000 trophozoïtes/μl and cultured in presence of antimalarial drug and incubated in 5% CO_2 for 48 h. These patients were treated at the inclusion.
RESULTS A total of 40 blood samples were collected. We obtained, 2.78% resistant isolates to quinine, 6.06% to monodesethyl amodiaquine and 52.94% to chloroquine. The geometric mean IC50 of lumefantrin, dihydroartemisinin and piperaquin were respectively 30.61, 1.31 and 8.58 nM.
CONCLUSION At the end of this study, we conclude that five (05) years after the adoption of policy for use of ACT in the treatment of uncomplicated malaria in Burkina Faso, there is a lower rate of *in vitro* resistance to quinine. Regarding dihydroartemisinin, there is no great change in the geometric mean IC50 values. And finally, we have a good antiplasmodial activity for monodesethyl amodiaquine, lumefantrin and piperaquin.
KEY WORDS *Plasmodium falciparum*, *in vitro*, Burkina Faso

1.5.5 Pathogenesis of severe malaria

P.1.5.5.001 (B)

Consequences of HIV infection on malaria clinical course and therapeutic implications in a population of Mozambique

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INTRODUCTION Some studies has demostrated that cotrimoxazol prophylaxis and antiretroviral therapy with protease inhibitors (PI) reduce parasitaemia and improve clinical outcome in patients with malaria and HIV co-infection.
AIM To compare the clinical severity of malaria in HIV-patients with the treatment administrated.
METHODS In a prospective, cross-sectional study, clinical and laboratory data were registered consecutively for all adults and children HIV-positive admitted to a medical ward in the Carmelo Hospital of Chokwe, Mozambique, during a period of 2 months (October–November 2012). HIV-positive patients with malaria diagnoses based in positive malaria blood slides were included.
RESULTS Forty-one patients were studied. Median age was 30.85 ± 16.69 years, 63.4% male. Average CD4 was 326 ± 316 and viral load 90 431 ± 21 916. There were five patients with complications (four severe anemia and one cerebral malaria) all of them without PI. Three relapses were detected. Thirty six patients were treated with artemether-lumefrantina and the remained with quinine. Any patients (0%) with cotrimoxazole prohylaxis had a parasitemia level of +++ or ++++ vs. 21.7%, and an average of days with fever of 2.57 ± 0.97 vs. 3.79 ± 2.64 (95% CI 0.024–2.42) compared with patients not taking cotrimoxazol prophylaxis ($P < 0.05$ in both). No relapses were reported in them. HIV treated with PI had this laboratory parameters in comparison with not treated: haemoglobin (g/dl): 9.1 ± 4.19 vs. 7.9 ± 3.56 ($P = 0.07$), leukocytes (μ/l): 5968 ± 5813 vs. 4099 ± 3293 ($P = 0.89$), Lymphocytes (μ/l): 1627 ± 1786 vs. 1338 ± 885 ($P = 0.59$), Platelets ($\times 10^3$ μl): 280 ± 193 vs. 236 ± 143 ($P = 0.42$). No complication was registered in these patients.
CONCLUSIONS The use of cotrimoxazole prophylaxis decreases the level of parasitemia and the duration of symptoms and prevents the relapses in patients with malaria and HIV infection. Patients treated with PI had no complication and tended to maintain higher levels of hemoglobin, leukocytes, lymphocytes and platelets, although the values were no significative.

P.1.5.5.002 (B)

Identification of a minimal group A PfEMP1 ICAM-1 binding site

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The *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP-1) plays an important role in antigenic variation and pathogenesis of malaria infections and can be classified into three major groups A-C sharing limited serological cross-reactivity. The 3D7 group A PFD1235w PfEMP-1 has been associated with severe malaria and erythrocytes infected with parasites expressing PFD1235w bind to ICAM-1, a phenotype linked with the development of cerebral malaria. We have previously identified pfd1235w-like genes and found these to encode a DBLa1.4-CIDRa1.6-DBLb3 domain cassette (DC4) of which the DBLb3 domains has been shown to bind ICAM-1. To identify the molecular determinants of adhesion, we used 3D protein modeling, created and expressed hybrid and substituted protein molecules and used these in ICAM-1 binding assays. Using this approach we identified a minimal PfEMP-1 binding epitope necessary and sufficient for the PFD1235w::ICAM-1 interaction. These results might have implications for the identification of parasites with an ICAM-1 binding phenotype and for the development of therapeutic interventions targeting such malaria parasites.

P.1.5.5.003 (B)

Characterisation the binding of *Plasmodium falciparum* isolates that express ICAM-1-binding DBLβ domains

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Adhesion between *Plasmodium falciparum* infected erythrocytes (IE) and different receptors on endothelial cells is a key factor in malaria pathogenesis. ICAM-1 binding by IE is thought to be associated with cerebral malaria. Several studies have identified some DBLβ domains as ligands for ICAM-1. This study identified the binding phenotypes of five IT lineage isolates that encode ICAM-1-binding DBL-β domains in their PfEMP-1s to ICAM-1Ref and four mutant ICAM-1 proteins using static assays. Our data show that different strains have different binding patterns to ICAM-1 and the mutant proteins. The study also examined the inhibition

caused by four anti-ICAM-1 mAbs on the binding of IE to ICAM-1Ref under static conditions with variable inhibitory effects. The data acquired from these experiments emphasise the variation in the contact residues of ICAM-1 by different parasite isolates.

P.1.5.5.004 (B)

Development of monoclonal antibodies targeting the surface of *Plasmodium falciparum*-infected human erythrocytes

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Cytoadhesion of *Plasmodium falciparum*-infected erythrocytes to endothelial cells lining the micro vessels of host organs is mainly linked to severe malaria infection. Cytoadhesion is mediated through the binding of *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) to various host receptors including Intercellular Adhesion Molecule 1 (ICAM-1), which has a role in cerebral malaria. In our study, we aim to develop monoclonal antibodies (mAbs) that target the potential adhesive Duffy Binding Like (DBLâ) domains of PfEMP-1 from ICAM-1 binding parasites. mAbs were raised against four recombinant proteins namely rDBL13, rDBL27, rDBL31 and rDBL41. We developed thirteen distinct monoclonal and polyclonal hybridoma clones that secrete IgM-class antibodies capable of recognizing the immunizing rDBLâ proteins. Flow cytometry results indicated that at least five monoclonal and polyclonal antibodies significantly labelled the surface of erythrocytes infected with IT4var16, IT4var14, IT4var13 and IT4var1 strains. These data suggest that oligomeric IgM may be developped to interfere with the ability of parasitized erythrocytes (PEs) to sequester and thereby reduce the mortality rate of cerebral malaria infection.

P.1.5.5.005 (B)

Loss of knobs on *Plasmodium falciparum*-infected erythrocytes is linked to expression of a specific PfEMP1

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Introduction The virulence of *Plasmodium falciparum* malaria is related to the parasite's ability to evade host immunity through clonal antigenic variation and tissue-specific adhesion of infected erythrocytes. The *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family expressed on knobs on the infected erythrocyte (IE) surface is central to both. Differences in receptor specificity and affinity of expressed PfEMP1 are important for IE adhesiveness, but it is not known whether differences in PfEMP1 expression influences knob density. The aim of this study was to investigate the change in Knob density on the IE surface in relation to PfEMP1 expression.
Material and Methods IE from the same genetic background were selected by antibody biopannings to express three different PfEMP1s. The knob density was analysed by Atomic Force microscopy and correlated to PFEMP1 and KAHRP expression.
Results and Conclusions When the IT4 laboratory strain was selected to express either IT4VAR4 (VAR2CSA) or IT4VAR32b there was no change in knob density. On the other hand when they were selected to express IT4VAR60 they lost the expression of knobs on the IE surface. This loss of knob expression was reversible since they gained the original knob density after subsequent selection for IT4VAR4 expression. Our findings aid the understanding of the regulation of knobs and PfEMP1 expression and the interaction between *P. falciparum* parasites and the infected host.

1.5.6 Malaria in pregnancy

P.1.5.6.001 (B)

The COSMIC consortium: community-based scheduled screening and treatment of malaria in pregnancy for improved maternal and infant health

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A EU funded interdisciplinary research consortium, COSMIC, comprising partners from Medical Research Council (The Gambia), Centre de Recherches Entomologiques de Cotonou (Benin), Centre Muraz (Burkina Faso), Institute of Tropical Medicine (Belgium), Imperial College (UK), WHO – Special Programme for Research and Training in Tropical Diseases and Royal Tropical Institute (Netherlands) has recently started their activities. The consortium aims at implementing scheduled intermittent screening of pregnant women with RDT by Community Health workers (CHW) at community level and treat positive women with antimalarials (SST). CHWs will also encourage pregnant women to attend antenatal clinics (ANC) for other pregnancy-targeted interventions such as IPTp/SP, thereby improving its coverage. This approach combines existing IPTp/SP with SST at village level as an extension of home based management of malaria (HMM). This simple (diagnosis by RDTs) and low cost intervention capitalizes on an already existing interventions (HMM) to improve maternal and newborn health. The project aims at determining the added value of community SST of pregnant women implemented through the CHW involved in HMM (as compared to IPTp/SP alone implemented in health facilities). Objectives are: (i) to identify bottlenecks for implementation of SST by CHW involved in HMM; (ii) to determine the impact of introducing SST in pregnancy on the quality of HMM; (iii) to determine the impact of SST on ANC attendance and IPTp/SP coverage; (iv) to determine the impact of SST on LBW, anaemia and placenta malaria; (v) to estimate cost-effectiveness of the intervention, and 6) to formulate recommendations for possible implementation.

Further information can also be found at our website: www.cosmicmalaria.eu.

P.1.5.6.002 (B)

The effect of daily Co-trimoxazole in pregnancy in an area of change in malaria epidemiology

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Introduction There are currently no available options for IPTp other than SP which has documented increase in resistance. Daily Cotrimoxazole (CTX) prophylaxis has proven antimalarial effect and significantly improves birth outcomes in HIV infected women with a low CD4 count. However, first trimester short term

treatments with CTX have been associated with birth defects. In the second and third trimester, it might also have deleterious effects.
METHODS A phase 3 randomized trial was conducted. It was later discovered there was no risk of malaria after HIV negative pregnant women and HIV positive pregnant women with CD4 count ≥200 cells/μl who were randomized had taken either three doses of SP–IPTp or two single strength tablets of CTX prophylaxis daily. These women were followed up until delivery and their babies until 6 weeks of age.
RESULTS There were 280 HIV negative pregnant women equally randomized to the treatment arms. HIV positive pregnant women with CD4 count ≥200 cells/μl were 52, and randomised with 27 on CTX and 25, SP. There were 14 with CD4 count <200 cells/μl. The pregnancy outcomes of HIV negative mothers showed no difference in preterm deliveries ($P = 0.80$), still births ($P = 0.82$) and neonatal deaths ($P = 0.2$) between the two treatment arms. This was also the case with the infant outcomes (birth weight, $P = 0.44$). The pregnancy and infant outcomes for HIV positive women in either arm showed no observable differences, however, the numbers for these groups were much smaller.
CONCLUSIONS Exposure to CTX during the latter part of pregnancy may not be associated with increased safety risks. However, more studies with bigger sample size are recommended to explore the safety profile further and its role for malaria prevention in pregnant women both HIV infected and uninfected.

P.1.5.6.003 (B)

Community perceptions of malaria in pregnancy: results from a qualitative study in Madang, Papua New Guinea

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BACKGROUND Malaria is the leading cause of illness and death in Papua New Guinea (PNG). Infection during pregnancy with falciparum or vivax malaria, as occurs in PNG, has health implications for mother and child, causing complications such as maternal aneamia, low birthweight and miscarriage. This article explores local understandings of malaria and risk perception during pregnancy and attitudes towards malaria in pregnancy (MiP) interventions in PNG.
METHODS As part of a qualitative study in Madang, MiP, participatory techniques (free-listing and sorting) were conducted along with focus group discussions, in-depth interviews (with pregnant women, health staff and other community members) and observations in the local community and health facilities.
RESULTS Attitudes towards and knowledge of MiP, its risks, and prevention varied amongst respondents. Although there was a general awareness of the term 'malaria', it was often conflated with general sickness or with pregnancy-related symptoms. Moreover, many preventive methods for MiP were related to practices of general healthy living. Indeed, varied messages from health staff about the risks of MiP were observed. In addition to ideas about the seriousness and risk of MiP, other factors influenced the uptake of interventions: availability and perceived comfort of sleeping under insecticide-treated mosquito nets were important determinants of usage, and women's heavy workload influenced Chloroquine adherence.
CONCLUSION The non-specific symptoms of MiP and its resultant conflation with symptoms or pregnancy that are perceived as normal has implications for MiP prevention and control. However, in Madang, PNG, this was compounded by the inadequacy of health staff's message about MiP.

P.1.5.6.004 (B)

Local illness concepts and their relevance for the prevention and control of malaria during pregnancy in Ghana, Kenya and Malawi: findings from a comparative qualitative study

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BACKGROUND In sub-Saharan Africa, the burden of morbidity and mortality linked to malaria during pregnancy (MiP) is significant and compounded by its unclear symptoms and links with other health problems during pregnancy. Mindful of the biomedical and social complexity of MiP, this article explores and compares local understandings of MiP and their links with other pregnancy-related health problems.
METHODS A comparative qualitative study undertaken at four sites in three countries: Ghana, Malawi and Kenya. Individual and group interviews were conducted with pregnant women, their relatives, opinion leaders, other community members and health providers. Findings: MiP-related behaviours were also observed at health facilities and in local communities. Across the four sites, local malaria concepts overlapped with biomedically defined malaria. In terms of symptoms, at-risk groups, outcomes and aetiology of malaria during pregnancy, this overlap was however both site-specific and partial. Moreover, the local malaria concepts were not monolithic and their descriptions varied amongst respondents. The symptoms of pregnancy and malaria also overlapped but, for respondents, symptom severity was the distinguishing factor. Malaria was generally, though not universally, perceived as serious for pregnant women. Miscarriage was the most widely known outcome, and links with anaemia, low birth weight and congenital malaria were mentioned. Nonetheless, amongst many potential causes of miscarriage, malaria was not recognized as the most important, but rather interacted with other pregnancy-related problems.
CONCLUSIONS Given the overlap of common pregnancy problems with the symptoms of malaria, and the limited association of malaria with its main outcomes, a comprehensive ANC programme is the most appropriate strategy for the provision of health education, prevention and treatment for MiP. Variations in locally shared understandings of MiP must however be taken into account when designing and promoting MiP intervention strategies.

P.1.5.6.005 (B)

An evaluation of knowledge and practices regarding malaria prevention and treatment among pregnant women in Niger State, Nigeria

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INTRODUCTION Malaria is highly endemic and remains one of the most leading causes of morbidity and mortality in Nigeria

with the most vulnerable groups being below 5 years of age and pregnant women. However preventative measures such as the use of Intermittent Preventative Treatment (IPTs) and Long-Lasting Insecticide Treated nets (LLINs) is a tested and effective strategy adopted by Nigeria to reduce this burden in accordance with the Abuja declaration.
Method I conducted 40 in-depth interviews and five focus group discussions (total = 80 respondents) with pregnant women living in two communities with moderate intensity malaria transmission. Respondents were randomly selected within age bracket 15–45 years. Questions focused on health concerns and knowledge, attitudes, and behaviours regarding malaria prevention and treatment as well as traditional and modern approaches to malaria prevention and treatment.
Results Eighty percent of the respondents identified malaria as one of the most deadly health issues affecting pregnant women in the area. Seventy percent expressed some correct knowledge over malaria transmission and prevention. Fifty percent use LLINs and 50% complain of discomfort due to misconceptions. Almost all respondents knew treatment for malaria existed and where to access them. Forty percent expressed concerns regarding malaria treatment during pregnancy as it regards the health of the mother and baby. Thirty percent admitted using traditional prevention and treatment approaches.
Conclusion Despite the progress being made in reducing the burden of malaria among pregnant women, there are still some misconceptions and the use of traditional prevention and treatment methods. Effort should be made by the government and policy makers to increase the knowledge of effective malaria prevention and treatment methods especially in the rural areas.

P.1.5.6.006 (B)

Prematurity, intra-uterine growth retardation, low birth weight: risk factors in a malaria endemic area in Southern Benin

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Introduction In developing countries, prematurity and intrauterine growth retardation still hold a predominant place and have important consequences since they are the two main mechanisms inducing low birth weight (<2500 g). Ninety-five percent of LBW newborn occurs in developing countries. In these countries, 2/3 of LBW are due to IUGR and 1/3 to prematurity. Our objectives were (i) to describe the contribution of prematurity and intra-uterine growth retardation to low birth weight in a malaria endemic area; and (ii) to identify risk factors associated with preterm birth and IUGR, and to explore their impact on birth weight.
Methods A cross-sectional study was carried out in maternity wards of Tori Bossito (Southern Benin) between June 2007 and July 2008. At delivery women's characteristics and newborn's anthropometric measurements were assessed. Gestational age was estimated using the Ballard method; William's sex-specific reference curve of birth weight-for-gestational-age was used to determine IUGR. Analyses were performed using multiple logistic and linear regressions.
Results Five hundred twenty-six mother-infant pairs were enrolled. LBW (<2500 g), prematurity (<37 weeks) and IUGR accounted for 9.1%, 10.3% and 25.3% of the sample, respectively. Male gender was associated with a lower risk of prematurity (OR = 0.52, $P = 0.03$). Low maternal anthropometric status (OR = 2.42, $P < 10–3$), primiparity (OR = 1.89, $P = 0.02$) and male gender (OR = 1.74, $P = 0.01$) were associated with an increased risk of IUGR. Among the above risk factors only low maternal anthropometric status and primiparity were significantly associated with an increased risk of LBW and their effect on LBW was mediated by IUGR. Moreover low maternal anthropometric status, maternal short stature and primiparity reduced mean birth weight.
Conclusion IUGR was the main mechanism mediating the effect of risk factors on LBW. Maternal undernutrition, either short stature or low anthropometric status, was the most important of them. Nutritional interventions are required in order to break intergenerational transmission of malnutrition.

P.1.5.6.007 (B)

The pregnancy registry for Eurartesim® is a monitoring programme designed to identify women taking Eurartesim® for the treatment of malaria during pregnancy

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Introduction This pregnancy registry has been approved by the European Medicines Agency (EMA) and the competent authorities as required by local regulation is active in Belgium, France, Germany, Italy, The Netherlands, Spain and the UK. A pregnancy registry is a study that collects health information from women who take medicines whilst they are pregnant. Information is also collected on the baby.
Material and Methods Participation in this pregnancy registry will help to identify the relevant information about exposure to Eurartesim® for the treatment of malaria during pregnancy. Personal data will not be disclosed, all of the medical information will be protected by the data protection law.There are three ways to obtain further information: pregnancy registry synopsis;telephone and email enquiries to country contact. Purpose of pregnancy registry: This pregnancy registry is an active, prospective, voluntary surveillance programme. We encourage the physician to refer all cases of Eurartesim® administered for the treatment of malaria during pregnancy as soon as possible, to collect prospective medical information on patients and their babies by answering specifically designed questionnaires. Criteria for enrolment in this pregnancy registry: The following patients will be included in this pregnancy registry:

1 Women who receive Eurartesim® for malaria whilst pregnant.
2 Women whose partner has received any formulation of Eurartesim® for malaria.
3 Women who have been signed the informed consent form.

The following patients must not be included in this pregnancy registry:

1 Women who refuse to participate.
2 Women participating in another study at the time of this pregnancy registry

P.1.5.6.008 (B)

Efficacy and safety of Mafloquine as malaria intermittent preventive treatment in pregnancy (IPTp)in HIV-infected women receiving daily cotrimoxazole prophylaxis: results from a multicenter randomized placebo-controlled trial

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The current recommendation by the World Health Organization (WHO) to prevent malaria infection in pregnancy in areas of stable malaria transmission relies on the prompt and effective case management of malaria illness, the use of intermittent preventive treatment (IPTp) with sulfadoxine-pyrimethamine (SP) at each scheduled antenatal clinic (ANC) visit and the use of insecticide treated nets (ITNs). The spread of parasite resistance to SP and the significant overlap in some regions of malaria transmission and high prevalence of HIV infection have raised concerns about the medium and long-term use of SP for IPTp. The evaluation of alternative antimalarials for IPTp is thus urgently needed in HIV-infected women. Among all the current available alternative antimalarial drugs mefloquine (MQ) is the one that offers the most comparative advantages to SP. A randomized double-blind superiority clinical trial was conducted in three African countries (Kenya, Tanzania and Mozambique) to compare the efficacy of three doses of MQ as IPTp with 3-dose placebo-IPTp in HIV-infected pregnant women receiving CTX prophylaxis. Pregnant women were enrolled at the ANC and followed until the infants were 2-months old. All participants received a long-lasting ITN at recruitment. In total 1071 pregnant women were recruited from March 2011 to January 2012. The results on the efficacy and safety of MQ as IPTp shall be presented.

P.1.5.6.009 (B)

Asymptomatic malaria and related anemia among pregnant women in Kinshasa, Democratic Republic of the Congo

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Introduction The aim of this study was to determine the extent of asymptomatic *P. falciparum* infection and its related anemia in healthy pregnant women living in Kinshasa, Democratic Republic of the Congo, an endemic area for malaria transmission.
Material and Methods A cross-sectional study was conducted in pregnant women attending prenatal care consultations. Information on socio-demographic characteristics was collected using a questionnaire. *P. falciparum* infection was diagnosed using Rapid diagnostic test and microscopy and Hemoglobin concentration was determined at the same time.
Results The prevalence of asymptomatic malaria was found to be 21% and 27% using blood smear and the rapid test respectively. The mean parasite density was 1146/μl. Prevalence was significantly higher among single women (36.7%) compared to married women (18.0%) ($P = 0.001$). *P. falciparum* infection was significantly lower among women who spent last night under a bed net (15.1%) compared to women who did not (38.9%) ($P = 0.03$). However, the differences in the prevalence of asymptomatic malaria among groups of gestational ages (1st, 2nd and 3rd trimester) between gravidity and among women who possess lattice windows or not were negligible. Prevalence of anemia was 61.9% and it was significantly associated with asymptomatic malaria ($P < 0.001$).
Conclusion These alarming results emphasize the need to actively diagnose and treat asymptomatic malaria infection during prenatal care visits, regardless of Intermittent Preventive Treatment with SP, and to increase efforts in promoting the use of Insecticide Treated Nets in DRC.

P.1.5.6.010 (B)

Placental malaria and seasonal patterns in rural Burkina Faso

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Introduction Malaria in pregnancy (MiP) remains a major public health problem in endemic countries. Placental sequestration of *P. falciparum* parasites causes maternal anaemia and low birth weight (LBW). A better understanding of seasonal patterns of placental infection could help targeting control interventions in areas of seasonal transmission.
Material and Methods One thousand ninety nine placental biopsies collected between 2004 and 2006 in primi- and secundigravidae enrolled in a cluster randomized trial on malaria preventive strategies in rural Burkina Faso were analysed. Biopsies taken from the maternal side of the placenta were classified as either negative or infected (acute, chronic or past) according to the presence of parasites and haemozoin. Peripheral parasitaemia and packed cell volume (PCV) were assessed during pregnancy and at delivery.
Results The large majority of collected placentas (71.9%) had signs of malaria infection, most of them (56.2%) past infections, 12.1% chronic and 3.6% acute. Active infections (acute and chronic) were significantly more frequent during the high transmission season (July to December). The risk of placental infection was the lowest in women delivering at the end of the dry season (May to June), when malaria transmission is extremely low. Active and past placental malaria was associated with maternal anaemia (active OR 2.3; 95% CI 1.39; 3.92; past OR 1.5; 95% CI 1.01; 2.21) and LBW (active OR 3.08; 95% CI 1.95; 4.89; past OR 1.44; 95% CI 1.03; 2.00). Intermittent preventive treatment with Ý2 doses of sulfadoxine-pyrimethamine (IPTp-SP) reduced the risk of placental infection by 37% ($P = 0.001$) as compared to incomplete (1 dose) IPTp and weekly chloroquine. Risk of active infection was reduced by 65% ($P < 0.001$).
Conclusions In rural Burkina Faso, acute and chronic placental infections, associated with LBW and maternal anaemia, show highly seasonal patterns. To reduce the burden of MiP, high coverage with IPTp-SP is needed during the peak transmission season.

P.1.5.6.011 (B)

Placental malaria and its effect on pregnancy outcome in the Blue Nile State, Sudan

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Background Malaria is a major public health problem in Sudan. Each year, more than 1 million women become pregnant in Sudan, of those 750 000 are in areas of intense perennial, high seasonal transmission or in areas of irrigation. Maternal mortality in Sudan is 509/100 000. Malaria during pregnancy also impacts the infant's health, as a result of infection of the placenta and malaria-caused maternal anaemia, which both contribute to low birth weight (LBW), the single biggest risk factor for neonatal mortality A Safe motherhood survey in 1999 in Sudan found that 46% of infant mortality occurred during the neonatal period.

Methods Between January 2012 and February 2013, 717 women delivering at Damazeen Hospital and Rusiers Hospital the two largest health centers located in the Blue Nile State, Sudan were recruited. Peripheral malaria slides were taken prior to delivery; placental smears were taken at the time of delivery. Information on the newborn and placenta were recorded soon after birth including infant birth weight and placental weight determination of the outcome of pregnancy.

Results Based on detection of parasites in either peripheral or placental blood smears primigravida women were more infected (39.2% compared to multigravida women (22.9%) at delivery time ($P = 0.01$). Thirty five percent of newborns weighed <2.5 kg at delivery time. Generally, multgravidae women had babies with mean birth weight that is significantly higher than that of primigravhidae ($P = 0.03$), the same was true for the mean placental weights ($P = 0.017$). Placental malaria was significantly associated with pre-term delivery and intrauterine growth retardation ($P < 0.01$). Moreover, birth weight was significantly lower in mothers infected deliveries compared to non infected deliveries in both gravidity groups ($P = 0.03$).

Conclusion Placental infection in Blue Nile State is prevalent and infections were associated with poor pregnancy outcome: pre-term, still birth and low birth weight.

P.1.5.6.012 (B)

Incidence of malaria infection and related risk factors among pregnant women in rural Burkina Faso

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Background Pregnant women are at higher risk of malaria. Malaria in pregnancy is associated with maternal morbidity and mortality. Most information on the burden of malaria during pregnancy relates to the prevalence of infection at a given time point. There is little information on the incidence of malaria in pregnancy in Burkina Faso.

Methods This study was part of a randomized clinical trial investigating the effect of intermittent preventive treatment with sulphadoxine/pyrimethamine during pregnancy on maternal and foetal outcomes. Pregnant women were enrolled in two health centers (Koho and Karaba) in South-west Burkina Faso and followed daily until delivery. Blood samples were collected for detection of malaria infection by microscopy. We used Poisson regression to study malaria risk in this population.

Results Among 1243 women enrolled, the overall incidence rate (IR) of malaria infection was 37.4 per 1.000 women-months, with no difference between health centres, i.e. 35.9 per 1.000 women-months in Koho and 40.9 per 1.000 women-months in Karaba ($P = 0.35$). The incidence of malaria infection was significantly higher in younger women [incidence rate ratio (IRR) 1.84, 95% CI: 1.30–2.61], during the rainy season (IRR 1.84, 95% CI: 1.40–2.44) and among primigravidae (IRR 2.44, 95% CI: 1.79–2.75). After the first trimester, malaria incidence was higher in primigravidae than in multigravidae (IRR 3.57, 95% CI: 2.44–5.17).

Conclusion Malaria infection among pregnant women in rural Burkina Faso was marked by seasonal variation. Targeting preventive measures to the season of higher risk, and gravidity may be appropriate in this setting.

P.1.5.6.013 (B)

Prevention and management of malaria during pregnancy: findings from qualitative studies in Ghana and Malawi

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Background In endemic regions of sub-Saharan Africa, malaria during pregnancy (MiP) is a major preventable cause of maternal and infant morbidity and mortality. Current recommended MiP prevention and control includes intermittent preventive treatment (IPTp), distribution of insecticide treated bednets (ITNs) and appropriate case management. This article explores the social and cultural context to the uptake of these interventions at four sites across Africa.

Methods A comparative qualitative study was conducted at three sites in two countries: Ghana and Malawi. Individual and group interviews were conducted with pregnant women, their relatives, opinion leaders, other community members and health providers. MiP-related behaviours were also observed at health facilities and in local communities.

Results ITNs were generally recognized as important for malaria prevention. However, their availability and use differed across the sites. In Malawi, ITNs were sought-after items but there were complaints about availability. In central Ghana, women saved ITNs until the birth of the child and they were used seasonally in northern Ghana. In central Ghana, pregnant women did not associate IPTp with malaria, whereas, in Malawi and northern Ghana, IPTp was linked to malaria, but not always with prevention. Although IPTp adherence was common at all sites, whether delivered with directly observed treatment or not, a few women did not comply with IPTp often citing previous side effects. Although opinions of diagnostic testing were generally positive, women's experiences of malaria testing varied and in Ghana and Malawi, treatment was sometimes administered in spite of a negative diagnosis. Despite generally following the advice of health staff, personal experience, the

availability and accessibility of antimalarials influenced the MiP treatment that women received.
Conclusion Although social and cultural context is relevant, personal experience also plays an important role in non-adherence to preventive or curative MiP interventions, particularly IPTp.

1.5.7 ACTs and new generation anti-malarials

P.1.5.7.001 (A)

Monitoring the supply and demand for antimalarials and RDTs: an update on ACTwatch

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Brief Introduction In 2008 Population Services International (PSI) in partnership with the London School of Hygiene and Tropical Medicine (LSHTM) launched a 5-year multi-country research project called ACTwatch to monitor antimalarial supply and demand in seven malaria endemic countries in Africa and Asia. Nationally-representative surveys have been conducted in Benin, Cambodia, the Democratic Republic of Congo (DRC), Madagascar, Nigeria, Uganda and Zambia. Phase two of ACTwatch begins in 2013 with expanded scope to include Kenya and Tanzania and additional research areas.
Material and Methods On average, three rounds of outlet surveys into the retail market for antimalarials were conducted in each country. Outlet surveys covered both the public and private sector, including informal private outlets. In addition, two rounds of household surveys focused on treatment-seeking behaviour for fever among children under 5. A systematic analysis of the antimalarial supply chain was conducted in each country by LSHTM. Outlet survey results focus on the availability, price and market share for antimalarials, and the availability and price of rapid diagnostic tests (RDTs) for malaria.
Results ACTwatch provides a comprehensive picture of the antimalarial market in target countries. In 2011, 53% of private sector outlets in Nigeria and 23% in Benin stocked quality-assured ACT. In comparison, 92% of private sector outlets in Nigeria and 69% in Benin stocked ineffective chloroquine treatments. In Benin, 30% of private sector market share was chloroquine in 2011, compared to 17% quality-assured ACT.
Conclusions Given the well-documented role of the private sector as a treatment source in sub-Saharan Africa, timely data on the availability, price and market share for antimalarials and diagnostics can help inform programming and health promotion campaigns. The second phase of ACTwatch will provide researchers, donors, and governments detailed data on RDT and ACT availability, with the potential to inform programs and improve access.

P.1.5.7.002 (A)

Effects of GLP-1 analogues on the *in vitro* growth of *Plasmodium falciparum*

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Introduction *Plasmodium falciparum* malaria affects the lives of millions of people worldwide. Type-II diabetes is on the rise in endemic regions presenting a risk both for co-morbidity and drug-parasite interactions. Glucagon-like peptide-1 (GLP-1) analogues are presently a front-line treatment for type-II diabetes. This study investigates the effects of GLP-1 analogues liraglutide (LIRA) and exendin-4 (Ex-4) on *P. falciparum* growth.
Methods *P. falciparum* was synchronized and cultured in the presence of vehicle (PBS) or increasing doses of LIRA, Ex-4 and artesunate (ART) for 48-h. Parasitemia was enumerated by flow cytometry. Repeated-dosing was investigated by a 96-h culturing regime with daily media/drug exchange and included GLP-1 receptor antagonist (Ex-9-39). Parasitemia was enumerated every 24-h. Single-dose assay: high dose LIRA and ART were added for 24-h. Cultures were grown for 96-h in total with a daily media change. Development: Seperate ring and schizont cultures were treated with high dose LIRA for 24-h. Development was assessed with blood-smear light microscopy.
Results Forty-eight-hours: LIRA (0.1, 1, 10, 100 μg/ml) and Ex-4 (1, 100 μg/ml) did not affect parasitemia. ART and high dose LIRA (800 μg/ml) significantly reduced parasitemia. Ninety-six-hours media change: Growth did not differ with LIRA-0.1,1,10 or Ex-4-1,100. LIRA-100 had reduced parasitemia at 72 and 96-h and was not inhibited by Ex-9-39. LIRA-800 was reduced similar to ART from 24-h. Single-dose: LIRA-800 and ART killed all parasites whereas LIRA100 did not affect growth. Development: LIRA-800 arrested the development of ring parasites whereas schizonts were not affected: cultures progressed into ring stages. No affect was observed with LIRA-100 after 24-h.
Conclusions *P. falciparum* growth is not affected by GLP-1 analogues at physiological doses (0.1–10 μg/ml). Parasite growth is inhibited with daily doses of LIRA-100 after 72-h, potentially independent of GLP-1 receptor (no affect of Ex-4-100 or Ex-3-39). LIRA seems to affect primarily ring stage parasites.

P.1.5.7.003 (A)

Repeated treatment with fixed-dose artesunate-amodiaquine versus artemether-lumefantrine in Ugandan children under 5 years of age with uncomplicated malaria

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The safety and efficacy of the two most widely used fixed-dose artemisinin-based combination therapies, artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) are well established for single episodes of uncomplicated *Plasmodium falciparum* malaria, but the effects of repeated, long-term use are not well documented. This was a 2-year randomized, open-label, longitudinal, phase IV clinical trial comparing the efficacy and safety of fixed-dose ASAQ Winthrop® and AL for the treatment of uncomplicated malaria in children <5 years of age. Children in the catchment area of Nagongera Health Centre IV, Uganda with malaria due to *P. falciparum* were randomized 1:1 to receive oral ASAQ or AL in dose regimen following guidelines. Subsequent episodes of uncomplicated malaria were treated with the same medication. A total of 416 children were enrolled and experienced a total of 6033 malaria episodes (mean ± standard deviation, 15 ± 5; range, 1–26). For the first episodes of malaria, the PCR-corrected-cure rate for ASAQ (97.5%) was non-inferior to that for AL (97.0%). For subsequent episodes of malaria in which >100 children were enrolled (episodes

2–18), the PCR-corrected cure rates ranged between 88.1% and 98.9% per episode, with no clear difference between treatments. For all episodes, parasite clearance was 100% by day 3, and gametocyte carriage was nearly eliminated (<1%) by day 21. Treatment compliance was close to 100%. Adverse events were mainly reported during the first malaria episode, were most often related to the malaria infection or concomitant infection or injuries, and were similar between treatment groups. Anemia or neutropenia was observed in ≤0.5% of the children per episode and abnormal liver function test in 0.3–1.4%. All biological abnormalities resolved spontaneously with no recurrence despite high rates of treatment re-administration. In this study, ASAQ and AL were found to be similarly safe and effective for repeated, long-term use.

1.5.8 Malaria epidemiology: burden and causes

P.1.5.8.001 (A)

Referral from community-based treatment providers: evidence from a cluster-randomised trial of mRDTs at community level in two areas of high and low transmission in Uganda

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INTRODUCTION Community based malaria treatment programmes increase access to effective antimalarials to populations beyond the reach of public health facilities. Despite their success in improving access to malaria treatment there is limited evidence on the referral of sick children beyond the skills of community medicine distributors (CMDs). We examined referral within the context of a cluster-randomised controlled trial to evaluate the impact of RDTs used by CMDs, compared with presumptive treatment, in two settings of high (HT) and low malaria transmission (LT) in rural Uganda.
MATERIAL AND METHODS A total of 120 villages (379 CMDs) were randomised to use RDTs (intervention) or a presumptive diagnosis of malaria (control). All CMDs were trained how to give antimalarial treatment with ACTs, rectal artesunate pre-referral treatment and how to recognise severe and non-severe signs of illness requiring referral to a health facility. Reasons for referral from CMDs and patient compliance to referral were captured from CMD treatment registers.
RESULTS In total 12792 children under five were seen in the high transmission setting and 2507 in the low transmission. In both settings referral was more common in the RDT arm compared to the control arm (HT: 36% in RDT arm vs. 1% in control arm, LT: 69% in RDT vs. 13% in control). Non-severe signs of illness accounted for more than 50% of referrals in both settings. Referral completion was low in both sites (13%), but was higher in the RDT arm in the high transmission site (RDT 13% vs. Presumptive 4%).
CONCLUSIONS CMDs using RDTs are more likely to refer children compared to CMDs using a presumptive diagnosis. Referral completion however was poor, with <15% of all children completing referral. The use of RDTs was associated with an increase in the proportion of children completing referral in high transmission setting.

P.1.5.8.002 (A)

Malaria in school children: epidemiology, disease burden and control approaches in areas of highly seasonal transmission

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INTRODUCTION Malaria control has traditionally focused on pregnant women and children under 5 years, in whom the risk of malaria-related mortality is greatest. Yet recent studies in areas of high transmission have shown that older school-age children could also benefit from malaria control, with potential gains for both health and education. Less is known about the impact of malaria control in schoolchildren living in areas of highly seasonal transmission. To address this, a series of epidemiological studies and intervention trials were undertaken in Senegal and Mali with the aim to characterize the epidemiology and disease burden of malaria in school-aged children in areas of seasonal transmission and to evaluate possible control approaches with a view to informing the development of effective strategies to maximise the health and educational potential of schoolchildren.
METHODS The methods used included longitudinal cohort studies, transverse parasitological surveys, nested case-control studies, as well as randomized controlled trials. These epidemiological studies were undertaken in three sites of contrasting transmission intensity and seasonality within Senegal.
RESULTS Data will be presented on incidence of clinical malaria, prevalence of asymptomatic malaria, malaria-related anaemia and cognitive function, to characterise the impact of malaria in schoolchildren living in areas of highly seasonal transmission; and disease burden compared under differing conditions of malaria transmission intensity. The implications of the findings and prospects for malaria control in schoolchildren will be discussed.

P.1.5.8.003 (A)

Injections, cocktails and diviners: therapeutic flexibility in the context of malaria elimination and drug resistance in Northeast Cambodia

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INTRODUCTION Adherence to effective malaria medication is becoming increasingly important in the context of Cambodia's elimination targets and drug resistance containment. Although in Ratanakiri province (Northeast Cambodia) public sector health facilities are accessible to the local ethnic minorities, their illness itineraries often lead them to private pharmacies or to local diviners. We carried out a social science study to understand the different contextual factors that influence a local person's choice for malaria treatment.
METHODS The research design consisted of a mixed methods study, combining qualitative (in-depth interviews and participant observation) and quantitative methods (household and cross

sectional survey). The main outcomes were the perceived efficacy of the different treatments and the perceived availability of the providers, their respective utilization rates, and the modeling of therapeutic pathways.
RESULTS Three broad options for malaria treatment were identified: (i) the public sector providing ACTs based on Mefloquine + Artesunate; (ii) the private sector with treatment 'drug cocktails' and artemether injections; (iii) traditional treatment based on divination and ceremonial sacrifices. Treatment choice was influenced by the availability of treatment and provider, perceived side effects and efficacy of treatments, perceived etiology of symptoms, and patient-health provider encounters. Moreover, treatment paths proved to be highly flexible, changing mostly in relation to the perceived efficacy of a chosen treatment.
CONCLUSIONS Despite good availability of anti-malarial treatment in the public health sector, attendance remained low due to both structural and human behavioral factors. The common use of underdosed anti-malaria monotherapy in the private sector (single-dose injections, single-day drug cocktails) represents a threat both for individual case management and for the regional plan of drug resistance containment and malaria elimination.

P.1.5.8.004 (A)

Socio-cultural factors contributing to the use, non-use and alternative use of topical repellents by indigenous populations in Northeast Cambodia

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INTRODUCTION The effectiveness of topical repellents in addition to long-lasting insecticidal nets is currently being evaluated in the forested and remote province of Ratanakiri, Cambodia. The introduction of repellents is meant to tackle residual malaria transmission induced by early and outdoor biting vectors.
METHODS A mixed methods social science study, combining qualitative ethnographic field research and a quantitative cross-sectional survey, investigated the acceptability and use of the topical repellents, ancillary to the epidemiological trial aiming at raising evidence on their effectiveness at community level.
RESULTS Self-reported daily use was estimated at 52.4%. Repellent use at times when anopheles mosquitos are active (in early morning and evening) was highest while people stayed in the forest (68.0%), and lowest in the villages (35.0%). At the slash and burn farms in the forest, 43.0% of respondents stated to always use the repellent in the early morning and evening; at the rice fields this was 42.3% of respondents. Alternative uses (including spraying on bed nets, on insects, on hair, etc.) were reportedly carried out by 88.0% of the population. The following categories of factors contributed to the use/non-use of the repellents: (i) acceptability factors: perceived direct and complementary benefits of the repellent (i.e. insect nuisance), perceived inconveniences (bad smell, skin irritation) and risks (perceived toxicity); (ii) accessibility factors (prolonged stays at farms in the forest during the rainy season); and, (iii) social organization (child care by older siblings prevented timely application of the repellent).
CONCLUSIONS Reported repellent use was highest among male adults performing work in the forest (i.e. slash and burn agriculture, hunting, logging) where insect nuisance was high. Possible social desirability bias, however, was considered a study limitation, leading to the development of a second phase of research using respondent independent methods (research ongoing).

P.1.5.8.005 (A)

Detecting foci of malaria transmission with school surveys: a pilot study in the Gambia

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BRIEF INTRODUCTION In areas of declining malaria transmission such as in The Gambia, the identification of malaria infected individuals becomes increasingly harder. School surveys may be used to identify foci of malaria transmission in the community.
MATERIALS AND METHODS A survey was carried out in May–June 2011, before the beginning of the malaria transmission season. Thirty two schools in the Upper River Region of The Gambia were selected with probability proportional to size; in each school approximately 100 children were randomly selected for inclusion in the study. A finger prick blood sample was collected from each child for the determination of antimalarial antibodies by ELISA, malaria infection by microscopy and PCR, and for haemoglobin measurement. In addition, data on socio-demographic variables and the use of insecticide-treated bed nets was collected using a questionnaire. The cut-off for positivity for antimalarial antibodies was obtained using finite mixture models. The clustered nature of the data was taken into account in the analyses.
RESULTS A total of 3277 children were included in the survey, with a mean age of 10 years (SD = 2.7) [range 4–21] with males and females evenly distributed. The prevalence of malaria infection as determined by PCR was 13.6% (426/3124) [95% CI = 12.2–16.3] with marked variation between schools (range 3–25%, $P < 0.001$), while the seroprevalence was 7.8% (234/2994) [95%CI = 6.4–9.8] for MSP119, 11.6% (364/2997) [95% CI = 9.4–14.5] for MSP2, and 20.0% (593/2973) [95% CI = 16.5–23.2) for AMA1.
CONCLUSIONS This survey shows that malaria prevalence and seroprevalence before the transmission season were highly heterogeneous.

P.1.5.8.006 (A)

Determining the predictors of awareness-raising activities for malaria prevention, among microscopists in Palawan Island, The Philippines

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BRIEF INTRODUCTION Palawan island is one of the most endemic areas of malaria in the Philippines, where health care

services are still limited. Toward the elimination of malaria in the island by the year 2020, prevention of the disease should be effectively conducted besides its early diagnosis and prompt treatment. The present study was conducted to identify the predictors of awareness-raising activities which are indispensable for malaria prevention, among microscopists (community health workers) who are currently most effectively working in Palawan island.

MATERIAL AND METHODS We performed a cross-sectional study among 127 (37% of 344) microscopists in Palawan island. The data were collected by using self-administered questionnaires from November 2010 to February 2011. Based on the results of descriptive analysis, Structural Equation Modelling (SEM) was conducted with the following items: (i) residential areas of the microscopists, (ii) Annual Parasite Index of the respective area, (iii) their capacity (service quality, knowledge of malaria, and microscope usage ability), (iv) malaria self preventive measures, and (v) job satisfaction of them.

RESULTS AND CONCLUSION In the SEM, microscopists' 'capacity' was found as a significant predictor of awareness-raising activities for malaria prevention. Their capacity was significantly explained by its two sub-components: their 'service quality' and 'microscope usage ability'. 'Job satisfaction' also explained their capacity, but it did not affect awareness-raising activities for malaria prevention. Thus in conclusion, strengthening of their service quality and microscope usage ability is the highest priority which will, in consequence, lead to the elimination of malaria in Palawan island.

P.1.5.8.007

Genotyping of *Plasmodium vivax* strains from Greece during the 2011 and 2012 outbreaks

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Greece was declared malaria free in 1974. Until 2010, about 35 malaria cases per year were reported, most of which, were travel-related while only few sporadic autochthonous cases have been reported. In 2011 however, a total of 96 malaria cases was recorded of which 42 were autochthonous; 36 of them derived from the district of Laconia. During 2012, 87 malaria cases were reported, but only 18 were autochthonous out of which, 10 were derived from Laconia. We genotyped the available *P. vivax* isolates to enhance the findings of classical epidemiology and elucidate the possible origin and spread of the disease.

Thirty-eight *P. vivax* isolates from 2011 and 71 isolates from 2012 cases were genotyped in the PvMSP-3α gene. All the 2011 isolates were further characterized using the microsatellites (MS) m1501 and m3502.

In Laconia in 2011, 14 of the samples expressed the A10-128-151 and 7 the A10-121-142 haplotype among the 29 samples studied which appeared throughout the period in autochthonous and imported cases. In other parts of Greece the haplotypes were largely unique.

Preliminary data from 2012 indicate that among the 32 samples derived from Laconia, 18 were of the A10 type while new PvMSP-3α profiles were found among the imported cases. The imported and autochthonous cases from elsewhere in Greece were again largely unique, with the exception of a PvMSP-3α profile (C3), which appeared in various regions.

The identical haplotypes among imported and autochthonous cases in Laconia indicate continuous transmission. The appearance of the same PvMSP-3α profile (A10) among both 2011 and 2012 isolates may indicate persistence of the same strain. However, this remains to be tested by ongoing MS analysis. The high genotypic variability among the imported cases indicate the multiple sources of malaria infection present in Greece, thus requiring vigilance of the local authorities to prevent malaria transmission.

P.1.5.8.008 (A)

Malaria-related morbidity and mortality in the Danga Health Area (Eastern Province, Democratic Republic of Congo)

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INTRODUCTION With more than 9 million cases in 2011, malaria is the primary cause of morbidity in the Democratic Republic of Congo. Due to an unusually high number of reported malaria cases and deaths, Médecins Sans Frontières implemented an emergency program in the Eastern Province in 2012. We performed a survey to estimate the retrospective mortality, the malaria prevalence, the hemoglobin level and the impregnated mosquito net coverage in the Danga Health Area.

METHOD An exhaustive, cross-sectional prevalence and retrospective mortality survey took place from 5 to 25 September 2012 in 874 households (4958 individuals). The recall period was 1 January – 25 September 2012.

RESULTS During the recall period, 128 deaths were reported (crude mortality rate = 1.1/10 000 persons/day); the under five mortality rate was 2.91/10 000 persons/day. The malaria-specific mortality rate was 0.79/10 000 persons/day. Among 4448 persons tested, 2111 (47.5%) had a positive rapid diagnostic test. Among children 0–4 years old, 38.9% had a positive RDT. Thick and thin blood smears were performed on 581 samples; 17.9% were positive. The average hemoglobin level was 11.6 g/dl (standard deviation = 1.7). Household mosquito net coverage was 56.2% and 99.7% of nets were impregnated.

CONCLUSION The high mortality, the high RDT positivity and the substandard mosquito net coverage suggest a worrying malaria situation in the Danga Health Area. Malaria control measures need to be strengthened.

P.1.5.8.009 (A)

Fighting malaria in a highly endemic region of the Republic of Guinea; measuring program impact

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BACKGROUND *Plasmodium falciparum* malaria is highly endemic and the primary cause of morbidity and mortality in Guéckédou Prefecture, Republic of Guinea. Since 2010 Médecins Sans Frontières Switzerland (MSF) in collaboration with the Ministry of Health has been implementing a multi-component

malaria intervention in one urban and two rural zones, with the objective of reducing malaria related morbidity and mortality. The aim of this study is to monitor the epidemiological trends of malaria in the intervention area (IA) compared to a control area (NIA) thus measuring the impact of the intervention.
METHODS Two cluster randomized cross-sectional surveys were conducted in the three zones of the IA (two rural and one urban) and one rural zone in the NIA, during the dry season. The first survey, conducted in April 2011, served as baseline, the second was conducted in February 2012 after 10 months of intervention.
RESULTS In total 13 894 individuals were surveyed. At baseline, according to rapid diagnostic test, malaria prevalence was 60.8% (95%CI: 58.3–63.3) in the IA and 64.5% (95%CI: 62.0–66.9) in the NIA. After 10 months there was a significant reduction in prevalence in the IA to 49.8% (95%CI: 47.0–52.6) while in the NIA the prevalence, 63.7% (95%CI: 60.8–66.7), was not significantly different from baseline. The decrease in malaria prevalence in the IA, when stratified by zone, was statistically significant in the urban and only one rural zone ($P < 0.001$ & $P = 0.003$).
CONCLUSIONS Our data from this on-going project, demonstrate for the first time, malaria prevalence in this highly endemic region. After 1 year, there was a significant reduction in the prevalence of *P. falciparum* in two intervention zones, which was not seen in the NIA. Although malaria prevalence remains unacceptably high, we believe the reduction in prevalence in the IA is very due to the MSF malaria control intervention.

P.1.5.8.010 (A)

Respondent-driven sampling on the Thailand-Cambodia border. II. Knowledge, perception, practice and treatment-seeking behaviour of migrants in malaria endemic zones

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BACKGROUND Population movements along the Thailand-Cambodia border, particularly among highly mobile and hard-to-access migrant groups from Cambodia and Myanmar, are assumed to play a key role in the spread of artemisinin resistance. Data on treatment-seeking behaviours, knowledge and perceptions about malaria, and use of preventive measures is lacking as characteristics of this population prevent them from being represented in routine surveillance and the lack of a sampling frame makes reliable surveys challenging.
METHODS A survey of migrant populations from Cambodia and Myanmar was implemented in five selected rural locations along the Thai-Cambodian border using respondent driven sampling (RDS) to determine demographic characteristics of the population, migratory patterns, knowledge about malaria, and health-care -seeking behaviours.
RESULTS The majority of migrants from Myanmar are long-term residents (98%) with no plans to move back to Myanmar, understand spoken Thai (77%) and can therefore benefit from health messages in Thai, have Thai health insurance (99%) and accessed public health services in Thailand (63%) for their last illness. In comparison, the majority of Cambodian migrants are short-term (72%). Of the short-term Cambodian migrants, 92% work in agriculture, 18% speak Thai, 3.4% have Thai health insurance, and the majority returned to Cambodia for treatment (45%), self-treated (11%), or did not seek treatment for their last illness (27%).
CONCLUSION Most highly mobile migrants along the Thai-Cambodia border are not accessing health messages or health treatment in Thailand, increasing their risk of malaria and facilitating the spread of potentially resistant *Plasmodium falciparum* as they return to Cambodia to seek treatment. Reaching out to highly mobile migrants with health messaging they can understand and malaria diagnosis and treatment services they can access is imperative in the effort to contain the spread of artemisinin-resistant *P. falciparum*.

1.5.9 Malaria diagnosis

P.1.5.9.001 (B)

Identification of genes differentially expressed in *Anopheles gambiae* salivary glands in response to infection with *Plasmodium berghei*

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The *Anopheles gambiae* mosquitoes are considerable one of the most important malaria vectors in Africa. Genomic and proteomic tools have been used to improve knowledge on the biology of mosquitos, providing relevant information to malaria control. The salivary glands (SG) have a central role in the parasite-vector-host interactions being fundamental not only in feeding but on pathogen transmission as well. The goal of this study is to identify and functionally characterize genes differentially expressed in the SG of *A. gambiae* mosquitoes in response to *Plasmodium berghei* infection. Three days-old *A. gambiae* mosquitoes were fed on mice uninfected abd infected with *P. berghei*. After 18 days, the SGs were removed for RNA extraction. The identification of genes was obtained by using direct RNA sequencing (RNA-seq) in an Illumina® GA IIX platform. The obtained reads were subsequently aligned using the software Bowtie (2.0) and then assessed by using the differential expression DESeq® program. After RNA-Seq, and using the *Z* test Kal with $P < 0.05$, 2623 genes were found to be differentially expressed, from which, 1594 genes were significantly overexpressed and 1029 downexpressed in infected mosquitoes. Information on genes found to be differentially expressed in *A. gambiae* SG after *P. berghei* infection results in a valuable tool for future studies. After confirmation of differential expression by real-time RT-PCR, gene knockdown assays will allow to enlarge our knowledge on their role in malaria infection and contribute to disease control.

P.1.5.9.002 (A)

Detection of asymptomatic infections caused by Plasmodium in blood donors using molecular platforms applied to pooled samples

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The impact of transfusional malaria represents a challenge for blood banks due to the increasing number of individuals moving globally. Despite the advances in blood screening aiming a safe transfusion, the choice for a reliable test is not simple, since is necessary to consider sensitivity, specificity, cost and applicability in large amount of samples. Data on the frequency of transfusional malaria show values from 0.2/million units of blood in non-endemic countries to 50 in areas with transmission. In our experience we detected several cases of symptomless donors with low parasitemias only detected by PCR that had transmitted infections. In a previous study we found that assays using pools are comparable to the individual analysis, and could be applied in hemotherapy services. Here we describe advantages of real time PCR protocols, reducing the processing time and cost. A total of 9280 blood samples from 147 Brazilian blood banks were arranged in 10 samples/pool. Real-time PCR was applied for genus-specific amplification targeting the ssrRNA 18S gene. Reaction was prepared with TaqMan® Master Mix (Applied Biosystems), primers M60/M61 and probe M62 marked with FAM™ and TAMRA™, assayed in duplicate. The second protocol used the same primers assayed with EvaGreen (Biotium) reagents. From the 928 pools tested by Real Time PCR, the TaqMan protocol detected 30 positive, with Cts lower than the cut-off (37.28). The EvaGreen protocol showed DNA amplification in 23 pools. Our results showed that 3.2% of pools assayed by real-time PCR amplified Plasmodium gDNA. The performance of this technique on pooled samples was similar when compared to the individual processing of our previous study. The earlier results show the superiority of TaqMan probe when compared to the EvaGreen protocol, which is less expensive. As this PCR is being standardized, the performance could be improved by means of changes in reaction parameters.

P.1.5.9.003 (A)

Incremental cost-effectiveness analysis of introducing rapid diagnostic testing for malaria into registered drug shops in Uganda

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Introduction Universal access to diagnostic testing for malaria followed by artemisinin-based combination therapy (ACT) for positive cases is now recommended by WHO for all treatment providers. It is common in Uganda for people to seek treatment for malaria outside the formal health care sector often with drug shops as their first point of care. Parasitological diagnosis to guide malaria treatment is not usually offered in drug shops.

Materials and Methods A recent cluster-randomised intervention trial in registered drug shops in Mukono District, Uganda, demonstrated that testing with malaria rapid diagnostic tests (mRDTs) in drug shops is feasible, can be associated with high provider compliance and can result in a significant increase in appropriate treatment compared to presumptive treatment. The incremental cost-effectiveness analysis of this intervention was evaluated using a decision analytical approach and supplemented with extensive sensitivity analysis. Provider costs per study arm incorporated the cost of community sensitisation before the interventions, training of drug shop vendors, development of training material and job aids; and the commodity costs such as the mRDTs used and the ACTs dispensed. The effectiveness measure was 'correctly treated patient' defined as a research blood slide positive patient receiving an ACT or a blood slide negative patient not receiving an ACT. Effects were also translated into Disability-Adjusted Life Years lost.

Results The incremental cost and effects of introducing mRDTs to increase appropriate ACT treatment in private, registered drug shops will be presented followed by sensitivity analyses incorporating factors like adherence, provider compliance and accuracy of the test.

P.1.5.9.004 (A)

Accuracy of MRDT on the diagnosis of malaria infection in children under 5 years without fever in the health zone of Mont Ngafula1, a hyper endemic area for malaria in Kinshasa, Democratic Republic of Congo

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In endemic countries with limited resources, diagnosis of malaria is often based on clinical signs while the method is quite unreliable. Nowadays, parasitological confirmation through microscopy or malaria rapid diagnostic test (MRDT) is currently recommended for each suspected case. In the field, many obstacles are found for the accurate use of microscopy. Therefore, the use of MRDT is promoted as a good alternative in African countries. However, there is limited information about accuracy and predictive value of MRDT in population based surveys of malaria prevalence, where asymptomatic people are more likely to have lower parasitemia than in clinical settings. This study aimed to assess the accuracy of MRDT and the prevalence of malaria infection in children under 5 years of age through a cross sectional household based survey conducted in the high transmission settings of the Health Zone of Mont Ngafula1 during the dry season from April to August 2012. A total of 800 children aged 3–59 months were included. The sensitivity, specificity, positive and negative predictive values with their CI95% of MRDT using microscopy as the gold standard were respectively 93.5% (90.0–97.1), 81.1% (77.9–84.2), 60.6% (55.0–66.3) and 97.5% (96.2–98.9) in the overall study population. The prevalence of malaria was of 24.9% (CI 95%: 21.0–26.7) with microscopy and 38.1% (CI 95% 33.9–40.6) with MRDT. A disadvantage of the MRDT is that in opposition to microscopy it does not allow to establish a threshold within symptomatic patients to assess malaria related fever. This is a main issue in endemic areas like our study site where up to a quarter of the children had the infection without having the disease.

P.1.5.9.005 (A)

Detection of *Plasmodium* by real-time PCR in a cross sectional study conducted in a cohort of pregnant women living in areas of low endemicity of malaria in the State of Sao Paulo, Brazil

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Pregnant women and children are the main risk groups for malaria. *Plasmodium falciparum* in pregnant women has been widely investigated worldwide, but there are few data about the interaction with other species. *P. vivax* is responsible for most cases in Asia and in Central and South Americas, with reports of morbidity and anemia in pregnant women and underweight in children. In Brazil the majority of cases occur in the Amazon basin, but there is still transmission in areas covered by Atlantic Forest, where asymptomatic infections occur. In Sao Paulo State, about 10% of the autochthonous infections are asymptomatic. In the city of Juquitiba, Sao Paulo, outbreaks are recorded, with occurrence of *P. vivax* and *P. malariae*. This study is unprecedented in this region and aims to follow-up pregnant women each 3 months to verify the occurrence of Plasmodium in this immunosuppressed cohort through the gold standard method and an advanced molecular diagnostic tool. During a period of 5 months, 83 pregnant women were enrolled in a prospective cohort. Blood samples were collected each 3 months in vacuum tubes, and thick blood smears were confectioned. Real-time PCR was performed for detection of Plasmodium genomic DNA, targeting the ssrRNA 18S gene. The reactions assayed in duplicate were prepared with TaqMan® Master Mix (Applied Biosystems), primers M60/M61 and probe M62 labeled with FAM™ and TAMRA™. The genus-specific real time PCR detected Plasmodium DNA in samples of three pregnant women, with Cts lower than the cut-off (37.15). Thick blood smears examination showed one positive sample. This study shows that asymptomatic cases are still happening in areas of very low endemicity for malaria, and even more alarming is the fact of the occurrence in immunosuppressed cohorts, as the pregnant women. This study is ongoing. The casuistic will be increased and immunological parameters will be evaluated.

P.1.5.9.006 (A)

Performance of microscopy and RDTs in the context of a malaria prevalence survey in Angola. A comparison using PCR as the gold standard

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Introdution Malaria burden estimation through community-based surveys is essential to malaria control as it identifies low parasitaemia infections and asymptomatic carriers. Therefore, accurate diagnosis enables realistic prevalence estimations. Microscopy remains the gold standard in diagnosing Plasmodium infections, however the use of Rapid Diagnostic Tests (RDTs) its becoming trendy as it allows practical, prompt and reasonably priced diagnosis. We compare the performance of microscopy and RDTs in identifying *P. falciparum* infections in the context of a community survey using Polymerase Chain Reaction (PCR) as the gold standard.
Material and methods We used data from 3307 individuals (preschool and school-aged children and their mothers/caregivers), tested for *P. falciparum* infection by microscopy, Paracheck-Pf® and PCR. The performances [sensitivity, specificity, positive and negative predictive values (PPV and NPV)] of microscopy and Paracheck-Pf® were compared using the McNemar's test and the weighted generalized score chi-squared test for paired data.
Results PCR and microscopy have identified similar prevalences of *P. falciparum* (15.9%), also close to that identified by Paracheck-Pf® (16.3%). However, microscopy had significantly lower sensitivity (60% vs. 72.8%), specificity (92.5% vs. 94.3%), PPV (60% vs. 70.7) and NPV (92.5% vs. 94.8%) than Paracheck-Pf®. Additionally, the sensitivity of both techniques was lower amongst mothers (36.8% for microscopy and 43.7% for Paracheck-Pf®), when in comparison with their children (68.4% in 0–5 years-old and 60.6% in 6–15 years-old for microscopy and 80.4% in 0–5 year-olds and 76.5% in 6–15 year-olds for Paracheck-Pf®).
Conclusion Using PCR as gold standard, both microscopy and RDTs were associated with unsatisfactory performances. False negativity and positivity were frequent in both techniques and possibly linked with known technical limitations found in low parasite density settings, level of expertise of microscopists and/or persistent antigenicity from previous infections (for RDTs). However, RDTs performed better than microscopy, supporting their use in malaria field based surveys.

P.1.5.9.007 (A)

Accuracy of the malaria rapid diagnostic test SD Bioline® in symptomatic children versus non symptomatic

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Introduction Several rapid tests are marketed for malaria diagnosis. The detection of low parasitemia is one of criteria for a good rapid test, although in high transmission areas people may carry parasites at a certain level without being ill. This study aims to determine the discrimination level of parasite density between symptomatic and non symptomatic children and the related accuracy of of rapid tests.
Methodology A cross-sectional study was conducted in the high transmission health zone of Mont Ngafula 1, Kinshasa city and Lisungi health center located in the same area. Participants (12–59 months old) were tested using both rapid test SD Bioline and Giemsa stained blood smear. The history of fever was investigated in the two groups. The ROC curve and the accuracy of the rapid test were performed using Stata. Ethical approval was obtained.
Results Eight hundred and ninety two children were recruited, including 280 attending the health center for fever or history of fever and 612 healthy ones found in the community. The median age was 35.1 months with an interquartile range of 25.9 months. The male were 53%. The threshold of discrimination between symptomatic and asymptomatic was 5218 parasites/microliter. The corresponding sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of rapid test were respectively 94.3% (95% CI: 89.8–97.2), 79, 6% (95% CI: 75.7–83.2), 62.1% (95% CI: 55.8–68.1) and 97.5% (95% CI: 95.5–98.8) in the

community. However, in the health care settings, the Se, Sp, PPV and NPV were respectively 99.4% (95% CI: 96.8–99.9), 67.5% (95% CI: 58.9–75.2), 78.9% (95% CI: 72.7–84.2) and 98.8% (95% CI: 94.4–99.9). These values have not improved much with different thresholds.
Conclusion The rapid tests selection criteria should take into the account the discrimination threshold of parasite density to enable better management of malaria in areas intense transmission.

P.1.5.9.008 (A)

Development of multiplex real-time PCR for the detection of *Plasmodium* species in the BD MAX system

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Background There is a need for an automated real-time (RT) PCR for malaria, which is sensitive, cost effective and capable of performing large scale diagnosis. We have used a multiplex RT-PCR assay for detection and differentiation of *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* for 2 years. This is sensitive but requires additional resources, time and cost. The BD Max™ system is a new, fully automated assay system for commercial and user-developed *in vitro* molecular diagnostic tests. It combines cell lysis, nucleic acid extraction, PCR setup, amplification, and detection in a single machine.
Methods We have modified our multiplex RT-PCR to the BD MAX ExK DNA-1 kit and BD MAX DNA MMK (SPC) master mix kit and implemented it in the BD MAX system. The primers used in this PCR: Plasmo1: 5′-GTTAAGGGAGTGAAGACGATCAGA-3′; Plasmo2: 5′-AACCCAAAGACTTTGATTTCTCATAA-3′. We tested the performance of this method on EDTA-blood from malaria patients with microscopically confirmed malaria. Our study included 19 samples with *P. falciparum*, two with *P. vivax*, and one with *P. ovale*.
Results The modified multiplex RT-PCR detected *P. falciparum*, *P. vivax*, and *P. ovale* correctly in all cases. We did not receive samples with *P. malariae* and *P. knowlesi* and were thus not able to test these two Plasmodium species in this method. The whole procedure for the test including DNA retraction and PCR took 2 h and required one BD MAX ExK DNA-1 kit. The blood sample volume could be reduced to 25 μl and the method was sensitive and low cost in resources and time.
Conclusions The presently developed multiplex RT-PCR in BD MAX system can be used for malaria diagnosis in clinical laboratories.

P.1.5.9.009 (A)

SDC PlasmoDetect®: a diagnosis based on the multi-angle light scattering spectroscopy for the presence of *Plasmodium* sp. in red blood cells

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Introduction The diagnosis of malaria is a significant challenge in resource-limited nations. The current standard method for determining whether a patient has malaria is to carry out a peripheral blood examination using a Giemsa-stained thick and thin blood film smears. This test requires a laboratory setting with a trained microscopist for accurate diagnosis. Spectra Digital Corporation (SDC) platform has developed a system where a diagnosis can be carried out in minutes in the field without the use of a microscope. It relies on comparing the scattering profiles from patient's blood incubated with a dye that selectively stains the parasite. The differences between the profiles can be used to predict for the presence of the parasite.
Methodology The basis of a light scattering measurement is to compare some kind of a treatment against a control. In this case, the treatment is the addition of the dye-Giemsa stain. The addition of the dye results in an amplitude change to the peak centered about 4 degrees if the blood cells are infected. No changes are seen if the blood cells are not infected. Algorithms for interpreting the data have been developed so that a person unfamiliar with light scattering theory can interpret the results and treat the patient if they test positive for malaria parasite. The accuracy of this new malaria diagnostic tool will be assessed using the PCR as gold standard and compared to microscopy and rapid diagnostic tests.
Expected Results The accuracy assessment is ongoing into the Democratic Republic of the congo. First result will be published in few months.

P.1.5.9.011 (A)

Detection of parasite and host-specific proteins in the sera of individuals infected with *Plasmodium knowlesi*

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Introduction Plasmodium knowlesi is a human malaria parasite that causes high parasitemia, severe clinical symptoms and mortality. To date, conventional PCR is the most efficient technique to confirm the diagnosis of *P. knowlesi*. However, it is not suitable in cases of acute malaria.
Aim In an effort to increase the specificity of *P. knowlesi* diagnosis, this study intends to identify new infectious markers that may potentially be used in the development of new diagnostic test(s).
Methodology Serum from 15 newly diagnosed patients with *P. knowlesi* ($n = 9$) and *P. vivax* ($n = 6$), along with 23 healthy individuals as a control group were used in this study. 2-DE and Western blot assay were used to study the circulating antigens of parasite and host-specific proteins in knowlesi infected individuals. Comparisons were made between serum protein profiles of *P. knowlesi* and *P. vivax* infected subjects with the control group. Antigenic proteins were identified using immunoblotting with pooled sera and monoclonal anti- human IgM-HRP.
Result The host-specific protein AHS, STF, C3, HPX, ZAG, Apo-AI, HAP and ABG in malaria infection exhibited immunoreactivity in Western Blots. STF and HPX only showed antigenicity and specificity in knowlesi infection while HAP only showed antigenicity in vivax infections. In addition, six *P. knowlesi* antigens were detected, which are K15, K16, K28, K29, K30 and K38.
Conclusion The identification of these antigens highlights the potential of these proteins and apparently modified host-specific proteins, STF, HPX and HAP, to serve as infectious markers for *P. knowlesi*.

P.1.5.9.011 (A)

Comparative evaluation of different methods for diagnosis of malaria

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The diagnosis of Malaria disease is essential to provide early treatment and improve the prognosis of patients. Serological methods often fail in diagnosing new borns due to their altered immune system, resulting in a need for new diagnostic methods reliably working with sample from patients ranging from 0 to 12 month of age. Transfusion-transmitted Malaria is rare, but it may produce severe problems in the safety of blood transfusion and blood related products due to the lack of reliable procedure to evaluate donors potentially exposed to malaria. Microscopy, still considered as gold standard for diagnosing malaria, is time consuming and requires trained expertise. Moreover errors occasionally occur especially at low parasitaemia limiting its use in blood banking and screening of populations. PCR shows a high sensitivity even at low parasitaemia and can distinguish between different Plasmodium species but it is expensive and a state of the art laboratory is needed. ELISAs are known to be ideal for high throughput screening with a high sensitivity and specificity but also here trained personal and an equipped laboratory is needed. Line blots are often used as confirmatory tests since they provide a high sensitivity and specificity. There is nearly no lab equipment needed to perform this kind of assay. In addition blots can be used in automated processes for high throughput screening. In our study blots seem to be a good tool for diagnosing malaria in new borns. Here we compare several serological diagnostic methods in concern of assay performance, clinical relevance, usability in low equipped labs and ability for high throughput screening. For this purpose we collected samples from all over the world, including samples from newborns. We also evaluated the performance of several methods directly in endemic countries with samples of patients who presented with symptoms akin to malaria infection in local hospitals.

1.6 Antibiotic resistance in man and animals

P.1.6.001 (A)

Identification of *Pseudomonas aeruginosa* isolates producing SHV-type extended-spectrum Betalactamase among hospitalized patients in two teaching hospitals, Sanandaj

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Objective *Pseudomonas aeruginosa* is one of the most important causes of nosocomial infections and can acquire resistant to many antibiotics, including â-lactams. The aim of this study was to determine the distribution and antibiotic susceptibility patterns of extended-spectrum beta-lactamase (ESBL) type SHV producing isolates from hospitalized patients in two teaching hospitals, Sanandaj, Iran.
Patients and methods A total of 123 *P. aeruginosa* were isolated from various clinical specimens. The double-disk synergy test was performed on the isolates for the detection of ESBL. SHV gene was confirmed by PCR method.
Results The most resistant antibiotics tested against *P. aeruginosa* were ceftazidime (23.58%) and cefotaxime (30.48%). All the *P. aeruginosa* isolates were tested for ESBL production and 22 (17.89%) were found to be ESBL producers. Out of 123 strains, 12 (10.57%) were SHV positive. Multiple resistances antibiotics were often associated with ESBL-producing organisms. Nosocomial infection (OR = 2.14), days of hospitalization (OR = 14.34), ICU hospitalization (OR = 3.4), presence of catheter (OR = 3.63), use of antibiotics within previous 2 weeks (OR = 5.51) and use of ventilator (OR = 3.75) were risk factors for pseudomonas nosocomial infection SHV positive ESBL.
Conclusion The high relatively of SHV producing bacteria in nosocomial agents of concern their potential spread among patients. The increasing risk factors for spreading of SHV enzyme were nosocomial infection, days of hospitalization, ICU hospitalization, presence catheter and use of antibiotics.

P.1.6.002 (A)

Patients' accounts of self-care and health care consultations in selected facilities in Eastern Region, Ghana

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Introduction There is an increasing trend in the prevalence of antimicrobial drugs resistance globally and Ghana is no exception. Antibiotic use is documented as an important determinant of resistance. We explored the intricate interactions that often lead to antibiotic prescriptions by examining patients' accounts of prior treatment seeking and consultations in healthcare facilities in Eastern Region, Ghana.
Methods The study was qualitative conducted in six healthcare facilities in the Eastern region of Ghana in April and May 2011. Semi-structured exit interviews were conducted with 24 out-patients who were prescribed antibiotics. The interviews were transcribed verbatim, field notes incorporated and imported into NVivo 10 for analysis.
Results Twenty of the patients had self-medicated prior to reporting to the health facility in total taking 24 different actions. Fifteen actions involved the use of biomedicines, 5 had used antibiotics. Self-medication was an attractive care practice for patients even though 21 were insured under the national health insurance scheme. Patients claimed that self-management of symptoms is a central strategy which, in their own words, prevented them from 'falling ill'. Selection of biomedicines for self-care was usually based on previous illness experiences and clinical encounters, and included the penicillins, tetracyclines, sulphonamides, chloramphenicol and metronidazole. Clinical consultations were brief. Clinicians may not consider patient's self-care practices in prescribing as only five were asked about self-care. Four patients underwent laboratory assessment before prescription. None of the patients made direct demands for medicines, but noted that they would be disappointed if medicines were not prescribed. There was limited communication between patient and prescriber in terms of illness aetiology, diagnosis, treatment and prevention.
Conclusions A more open communication between clinicians and patients especially relating to disease aetiology, diagnosis, prevention, and self-care may have the potential to transform both prescribers' and patients' treatment practices and prevent unnecessary use of antibiotics.

P.1.6.003 (A)

Carriage of cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* in the Ghanaian community

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Background Development of antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae* is an acknowledged problem in western countries. This work presents the first prevalence study in Ghana on of fecal carriage of cephalosporin-resistant *E. coli* and *K. pneumoniae*.
Method Fecal samples from 'healthy' individuals in Korle-Gonno, Dodowa, and Tamale in Ghana were collected from March 2011 to January 2012. *E. coli* and *K. pneumoniae* isolates were cultured and screened by the combined disk synergy tests, examined for antibiotic susceptibility patterns, and cephalosporin-resistance- gene characterization.
Results During the study, fecal samples from 227, 215 and 294 individuals from the Korle-Gonno, Dodowa, and Tamale community, respectively, were collected. Among these, 135 (59.5%) from Korle-Gonno, 86 (40%) from Dodowa and 117 (40%) from Tamale had at least one cephalosporin-resistant *E. coli* or *K. pneumoniae*. A total of 141 cephalosporin-resistant isolates (139 *E. coli* and two *K. pneumoniae*) were recovered from Korle-Gonno, 136 *E. coli* had ESBL genes only, two *E. coli* and two *K. pneumoniae* had ESBL + AmpC genes and one *E. coli* strain produced carbapenemase. Of the 95 *E. coli* isolates from Dodowa, 89 produced only ESBLs, one both ESBLs and AmpCs, two only AmpCs and one strain produced carbapenemase. From Tamale, of 161 *E. coli* isolates 130 produced ESBLs. CTX-M enzymes were most prevalent in all three communities 98% (136) for Korle-Bu, 97% (92) for Dodowa and 88% (117) for Tamale, CTX-M-15 being the dominating ESBL.
Conclusion The prevalence of ESBL producing *E. coli* and *K. pneumoniae* the Ghanaian community was found to be 59.5% from Korle-Bu, 40% from Dodowa and 40% from Tamale with CTX-M-15 dominating. This is one of the highest ESBL-fecal carriage prevalences reported in the World.

P.1.6.004 (A)

Antimicrobial susceptibility and genetic diversity of clinical *Staphylococcus aureus* from health care institutions in Ghana

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Introduction Antibiotic resistance in *Staphylococcus aureus* continues to be major problem worldwide. In Ghana, data on antibiotic sensitivity patterns and clonal diversity of S. aureus is limited. Therefore, a programme under the Danish Ministry of Foreign affairs was set up to get an insight into the antibiotic susceptibility and genetic diversity of *S. aureus* in Ghana to enhance implementation of antimicrobial policies. In this study, antibiotic susceptibility and clonal diversity were determined in a collection of clinical isolates obtained between September 2010 and March 2012 from six health care institutions located across the Northern, Central and Southern areas of Ghana.
Methods *S. aureus* isolates were identified and confirmed by tube coagulase and staphplus test (Biomerieux); antibiotic susceptibility testing was done by disk diffusion method according to EUCAST guidelines.. The genes encoding methicillin resistance (mecA), staphylococcal protein A (spa), panton valentine leukocidin (luk-PV) were determined by multiplex PCR.
Results Of the 308 clinical isolates, 97% (n = 299) were resistant to penicillin, 42% (n = 128) to tetracycline, 6% (n = 17) to erythromycin and 3% (n = 9) to cefoxitin. Based on spa typing 99% (n = 305) isolates clustered into 14 MLST clonal complexes: CC377 (n = 80) CC15 (n = 55),CC121 (n = 41), CC8 (n = 36), CC5(n = 34), CC1(n = 28), CC88 (n = 8), CC45 (n = 8) CC30 (n = 4) CC9 (n = 3), CC25 (n = 2), CC97 (n = 2) CC20 (n = 2), and CC707(n = 2).The frequent spa types were t355 (19%), t084 (12%) and t314 (6%). MRSA found among isolates belong to seven major clones: ST88-IV (n = 2), ST8-IV (n = 1), ST789-IV (n = 1), ST72-V (n = 1), ST2021-V (n = 1), ST250-I (n = 2), and ST239-III (n = 1). Most isolates (60%) were pvl positive, majority of these were MSSA.
Conclusion In this first detail phenotypic and genotypic study of *S. aureus* from clinical infections, we found seven MRSA lineages one of which is related to USA300 (ACME+; pvl+) and high pvl prevalence among MSSA isolates from Ghana.

P.1.6.005 (A)

Prevalence and molecular characterization of cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates in Ghana

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Introduction The ADMER (Antibiotic Drug use, Monitoring and Evaluation of Resistance) programme was initiated to strengthen surveillance of antimicrobial resistance in Ghana. One of the objectives, was to report the prevalence, antimicrobial resistance and molecular characterization, and assess the risk predictors associated with infections caused by Beta-lactamase [AmpCs, Extended-spectrum Beta-lactamase (ESBL), and carbapenemases]-producing *Escherichia coli* and *Klebsiella pneumoniae* strains at the largest health facility in Ghana, Korle-Bu Teaching Hospital.
Methods From April through December 2011, non-duplicate isolates of *Escherichia coli* (199) and *Klebsiella pneumoniae* (37) implicated as causative agents of infections were prospectively collected. Strains were subjected to AmpC, ESBL and carbapenemase phenotype confirmation, PCR and gene sequencing. *Escherichia coli* strains were studied for phylogenetic groupings and typed for presence of 025b:ST131 clones. All patients were screened for intestinal colonization with cephalosporin resistant *Escherichia coli* and *Klebsiella pneumoniae*. A case-control study was performed using multivariate calculations for risk factors.

Results The isolates were mainly recovered from inpatients (66%; 155/236) with urinary tract infections (68%; 160/236). Overall, 59% (*E. coli*, *n* = 126; *K. pneumoniae*, *n* = 13) of 236 isolates demonstrated plasmid AmpC genes [3%; blaCMY-42 (*n* = 5), blaDHA-1 (*n* = 3)] or ESBLs (56%, *n* = 132) or metallo-beta-lactamases [0.4%, blaNDM-1/blaCMY- 42 (*n* = 1)]. Among ESBL strains, CTX-M-15 was most prevalent (*n* = 126, 96%). An allele-specific PCR for 025b:ST131 revealed a low prevalence (6%, *n* = 8/126) of ST131 clones that cluster within phylogroup D CTX-M-15-only strains. Multivariable analysis indicated intestinal colonization with beta-lactamase producing strains as significantly associated with infections among inpatients [odds ratio (OR), 1.3; 95% confidence interval (CI), 1.1–1.51; *P* = 0.01]. Each day of cephalosporin use within 1 year prior to culture date increased the risk of infection by 8% (OR, 1.8; 95% CI, 1.18–2.6.; *P* = 0.01).
Conclusion CTX-M-15 producing *Escherichia coli* constitute a significant problem in Ghana but ST131clones are rare. Intestinal colonization may contribute to its dissemination.

P.1.6.006 (A)

Unwanted souvenirs from travelling abroad – colonization with extended-spectrum beta-lactamase-producing *Enterobacteriaceae*

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Introduction Multidrug-resistant gram-negative bacteria, especially extended-spectrum beta-lactamase- (ESBL) and carbapenemase-producing Enterobacteriaceae, are an increasing public health threat worldwide. Travelling, especially to Asia, is known to be a risk factor for becoming colonized with these bacteria. However, little is known about the real extent to which travelling contributes to the global distribution of these bacteria.
Material and methods Travellers presenting themselves for pre-travel advice at two Swiss travel clinics before travelling to South Asia (India, Bhutan, Nepal, Sri Lanka, Bangladesh) were enrolled into the study. A faecal sample was obtained and a questionnaire was filled in before and directly after travelling as well as 3 months after returning from South Asia. The faecal samples were screened for extended- spectrum and other beta-lactamase-producing Enterobacteriaceae. The proportion of travellers newly colonized with multidrug-resistant Enterobacteriaceae after travelling was calculated for the different destinations and compared to each other, using a Chi-square test.
Results A total of 200 travellers will be included into the study. The results of the first 67 travellers who provided faecal samples before and after travelling to South Asia showed an overall incidence of becoming colonized while travelling of 80% (95% CI 68.2–88.9%). Significantly more travellers to India (87%) became colonized as compared to travellers to the other countries (63.2%, *P* = 0.029). Colonization rates 3 months after returning from South Asia appear to decline considerably.
Conclusions This study with the highest number of travellers to South Asia enrolled so far confirms the high incidence of travel-related colonization with ESBL-producing Enterobacteriaceae in travellers to South Asia, especially to India, found in previous studies. International travel seems to be a major contributor for the worldwide spread of multidrug-resistant gram-negative bacteria. Although the extent to which this is associated with clinical consequences remains unclear, the potential public health threat is considerable.

1.7.4 Neglected bacterial infections

P.1.7.4.001 (B)

The relationship between *Helicobacter pylori* infection and iron deficiency anemia among 2–12 year old children in Northwest of Iran

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Introduction *Helicobacter pylori* infection is a common human bacterial infection. It is estimated that about half of people throughout the world are infected by this gastric microbial pathogen. The outcome of *H. pylori* infection varies from gastritis to peptic ulcers and gastric malignancies. Recent several studies have shown that *H. pylori* infection has been associated with iron deficiency anemia (IDA) in children. The objective of this study was to estimate the prevalence of *H. pylori* infection and iron deficiency anemia and probable association between these two variables among 2–12 year-old children in Ardabil (Northwest of Iran).
Materials and Methods In this descriptive and cross-sectional study, we selected 960 children (2–12 years old) randomly, who referred to pediatric clinics in Ardabil (from 2011 February to 2012 February). Then we collected the blood and stool samples of them to asses *H. pylori* infection and iron deficiency anemia. *H. pylori* infection was evaluated using HpAT (*Helicobacter pylori* stool Exam Antigen Test) method, and for determining iron deficiency anemia, the seral levels of Hb, Si, Ferritin and TIBC were measured. Finally the data were analyzed by SPSS (version 18) software.
Results Of 960 studied children, *H. pylori* infection was present in 360 cases (37.5%), 140 cases of *H. pylori* infected children (38.9%) were boys and 220 cases (61.1%) were girls. Iron deficiency anemia was present in 80 children (7.3%) and only 25 children (6.9%) were positive for both *H. pylori* infection and iron deficiency anemia.
Conclusion In this study, we confirmed that the high prevalence of H. pylori infection persists among 2–12 year-old children in Northwest of Iran (Ardabil), and according to Fisher's Exact Test (á = 0.05) we found significant relationship between *H. pylori* infection and sex of the children (*P* = 0.001). But we found no significant association between H. pylori infection and iron deficiency anemia.

P.1.7.4.002 (B)

Frequency and drug resistance profile of bacteria isolated from urinary tract infection in ambulatory patient of Tehuacan-Puebla, Mexico

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Bacteriuria is a common term to refer presence of bacteria in urine and refer to a Urinary Tract Infection (UTI) which is a global ailment and México is not the exception. Hospitalized patients are exposed to many risk factors including the prophylactic overuse of antibiotics in about 20% of the hospitalized patients, but not the case of ambulatory patients. For that rea-

son, the objective of this work was to establish the frequency of UTI in all ambulatory patients collected in a private laboratory from private consult, and set up the more frequent bacteria isolated and its antibiotic sensitivity profile.
Methods Samples were collected from patient using the standard aseptically middle stream, the criteria of pure culture, the significant bacteriuria counts, and using selective and non-selective culture media. The identification and sensitivity antibiotic profile was made using the miniAPI®-32 system galleries and the miniAPI® semiautomatic lector from Biomerieux.
Results 199 strains were identified from 191 samples, eight samples had two different species of bacteria and all were evaluated for antimicrobial resistance profile. *Staphylococci* (25.76%) was the more frequently bacteria isolated from these patients followed by *Escherichia coli* (20.71%). Women ranging between 20–40 years (29.32%) and boys from 0 to 10 years (14.14%) were the most affected patients. Imipenem, Meropenem and Amikacin showed to be effective against gram negative bacteria. Fusidic acid, Nitrofurantoin, Vancomicin and Teicoplanin showed to be effective against *Staphylococcus*. In other few cases was isolated other genera like Corynebacterium (8.08%) Enterococcus (8.08%), Streptococcus (6.06%), Micrococcus (3.54%), Enterobacter (4.04%) and Pseudomonas (1.01%). However, some of them showed high resistant to many types of antibiotics.
Conclusions We conclude that is highly important to consider the antibiotic resistance increase by bacteria producing UTI and the importance to use the correct antibiotic according to the sensitivity antibiotic profile.

P.1.7.4.003 (B)

Urinary tract infection among intellectual disability individuals 'Etiology and antibiotic resistance patterns' in rehabilitation centers of Mazandaran province, Northern Iran

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Objective Urinary tract infections (UTIs) are amongst the most common infections and account for large proportion of antibacterial drug consumption. The aim of this study was to determine the rate and the etiologic agents of UTIs in inhabitants of rehabilitation centers of Mazandaran province in northern Iran and to evaluate the antimicrobial susceptibility patterns of the uropathogens isolated.
Methods Clean catch midstream urine sample was collected from each of 314 participants (163 males, 151 females) residing in 12 rehabilitation centers of Ramsar, Nowshahr, Chalous, Amol, Sari and Behshahr. Urine specimens were cultured and bacterial isolates were identified by conventional methods. All urines fulfilling the criteria for the presence of significant bacteriuria (≥104 cfu/ml urine) were defined as positive. Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method.
Results The rate of urinary tract infection was 30.9% with the highest rate in pediatrics ($P < 0.0001$).The prevalence of UTIs were shown to be higher in females than in males with the rate of 46.3% in young aged females (20–29 years), 60% in middle aged group (40–49 years) and 50% in elderly (>50 years). Bacteria most frequently isolated from urine specimens was *Escherichia coli* (39.2%) with the highest rate of infection in females age group <10 years ($P < 0.001$). Among the antibiotics tested against the isolated organisms for susceptibility test, ceftriaxone and gentamicin maintain good activity against the majority of gram negative bacteria that cause UTIs recovered from individuals with intellectual disability. Vancomycin was effective against *Staphylococcus aureus*.
Conclusions This survey shows that the prevalence of UTIs among inhabitants of institutions for mentally retarded persons in northern Iran and the susceptibility pattern of uropathogens to antibiotics are similar to many published studies conducted in normal populations.

P.1.7.4.005 (B)

The usefulness of filter paper spotted sera for leptospirosis surveillance in the Pacific

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Leptospirosis is a worldwide zoonosis most common in the tropics. The spectrum of the disease is extremely wide and misdiagnoses are frequent. Acute phase diagnosis relies on molecular testing. If not locally available it requires shipment of frozen samples to reference laboratories. Molecular diagnosis tools are often absents in low resources and remote areas and frozen shipment is not possible. Filter paper (FP) spotted serum or whole blood is exempted from dangerous goods regulations. In order to overcome the difficulties in the shipment of samples we developed a protocol for leptospirosis diagnosis by PCR from FP-dried serum or whole blood. We received samples from different South Pacific islands including Santa Cruz Island (Solomon Islands) and Yap State (Federated States of Micronesia). At the time of initial consultation, venous or capillary blood samples were collected and spotted on FP. The FP-dried samples were then sent to our lab (French Polynesia) by standard mail at ambient temperature. As our extraction protocol allows both DNA and RNA extraction, the same extracts were submitted to leptospirosis real-time PCR and dengue virus multiplex reverse transcription real-time PCR. On the 172 FP-dried samples collected from dengue suspected patients in Yap State, five were leptospirosis PCR positives. On the 37 FP-dried samples collected from Santa Cruz Island, also five were leptospirosis PCR positives (four had initially suspected malaria infection and one had suspected otitis). Using the same protocol we confirmed that dengue virus serotype 2 was still circulating inYap State. We previously demonstrated the interest of using FP-dried samples for dengue surveillance in the Pacific. This protocol is now validated for leptospirosis. It is a potentially powerful tool to enhance leptospirosis and other infectious diseases surveillance capacity in the Pacific and in other remote or resource-poor regions.

P.1.7.4.006 (B)

Analysis of virulence factors in *Escherichia coli* causing bacteraemia in Peruvian children

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Introduction *Escherichia coli* is one of the most common Gram-negative pathogen causing bloodstream infections in

paediatric patients in Peru. The disease's severity is determined by the presence of different virulence factors (VF). This study aimed to analyse the presence of several VF in *E. coli* blood isolates from several hospitals of Lima, Peru.
MATERIAL AND METHODS Sixty-five *E. coli* causing bacteraemia were isolated from children under 5 years old (71% from neonatal services). *E. coli* phylogroups and the presence of several VF (hly, sat, cnf, fimA, papC, shf, agn43, sepA, sigA, pet, pic and espC genes) were determined by PCR.
RESULTS Thirty (46%), 19 (29%) and 14 (22%) *E. coli* isolates classified into phylogroups D, A and B1, respectively, while 2 (3%) belonged to phylogroup B2. Isolates contained up to five VF, with a mode of three genes (24.6%). Despite the limited number of samples, the isolates from phylogroups D and B2 carried a greater number of VF comparing with A or B1 (3.2 and 3.5 vs. 1.7 and 2.2 respectively). The frequency of genes encoding for fimbriae was 89.4% for fimA and 18.2% for papC, whereas that of shf and the SPATE gene agn43 (biofilm-related genes) was 19.7% and 69.7% respectively. The remaining SPATES were less frequent: sat (25.8%), pic (4.5%), sigA (1.5%). No sepA, pet and espC genes were detected. Meanwhile, hly and cnf1 accounted for 12.1% and 6.1%, respectively. The known association between hly and cnf1 was found in only 50% of cases.
CONCLUSIONS The analysed fimbriae and biofilm related factors and the sat toxin are the most frequent VF in these isolates. Despite the low number of samples, the isolates belonging to the phylogroups D and B2 possessed a higher number of VF, although the relevance of the different phylogroups differs than those usually described, being the phylogroup B2 underrepresented.

P.1.7.4.007 (B)

Evaluation of the surveillance system of invasive meninigococcal disease in the greater Tunis by the capture-recapture method

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INTRODUCTION Invasive meningococcal disease is a serious and potentially life-threatening infection caused by the bacterium *Neisseria meningitidis*. Immediate recognition and treatment is critical. Reporting of cases is also crucial so that the proper control measures can be quickly implemented for prevention of secondary cases. Passive and active surveillance systems are used to monitor meningococcal disease, which is a notifiable disease in Tunisia. The objective of this study was to assess the completeness of the system of epidemiological surveillance of invasive meningococcal diseases in the greater Tunis.
PATIENTS AND METHODS A 6 year retrospective study (2003–2008) identified all invasive meningococcal disease cases appearing on Mandatory Notification records of the Regional Directorate of Health of Tunis and those reported by the microbiology laboratory of children's hospital of Tunis, considered as the main source of the invasive meningococcal disease diagnosis in the Greater Tunis. The 'capture-recapture' method was applied.
RESULTS During the study period, we analyzed 38 cases of invasive meningococcal disease reported to the Regional Directorate and 47 cases provided by the children's hospital. We identified 25 cases common to both sources. The total number of cases of invasive meningococcal disease was estimated at 71 [95% CI = (60–82)], using the capture-recapture method. Exhaustivity rates were 53.5% [95% CI = (46.6–62.9)] for Mandatory Notification to the Regional Directorate and 66.2% [95% CI = (57.6–77.8)] for children's hospital. Stratified analyses suggest that the two data sources were probably independent.
CONCLUSION The capture-recapture method applied to the surveillance system of invasive meningococcal disease in the Greater Tunis showed a poor sensitivity of the Mandatory Notification. Factors influencing the under-reporting of cases should be identified to enhance such surveillance.

P.1.7.4.008 (B)

Effective wound management under low resource conditions

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BACKGROUND Chronic wounds are a common medical problem in developing countries. Their epidemiology differs from industrialized countries with a much higher proportion of traumatic and infectious causes and a more even age distribution. Inadequate hygiene, insufficient arterial or venous blood circulation, mechanical irritation and malnourishment are major obstacles to wound healing.
RESEARCH QUESTION Remarkable success with a simple and cost effective treatment protocol can be achieved in the management of wounds of various aetiologies.
METHODOLOGY Case presentation.
RESULTS We present three large wounds, one caused by *Mycobacterium ulcerans* (Buruli Ulcer), one presumably by a snake bite and one by streptococcal infection (erysipela). The treatment protocol includes saline, povidone-iodine, sterile gauzes and elastic bandages only. In our observation this protocol produces good results when applied under strict hygienic conditions by a motivated team. Additional factors of relevance are a stable supply chain, adequate nutrition, pain relief, surgical debridement if indicated and physiotherapy. A (picture based) documentation is helpful to identify problems and provide feedback to carers and patient.
CONCLUSION Chronic wounds may be the most neglected tropical disease. Large improvements in management and outcome are possible with simple materials and techniques.

1.8 Tuberculosis, lack of resources or lack of attention?

P.1.8.001 (B)

Active tuberculosis in patients under therapy with TNF-α inhibitors

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Although tuberculosis screening and exclusion of active disease is mandatory prior to initiation of treatment with biologics, TNF-α inhibitors in particular, active diseases are reported worldwide in this context. Aim of our study-in a case series of eight patients with tuberculosis activated during treatment with

Table 1 Clinical manifestation of tuberculosis in 8 patients under therapy with TNF-a inhibitors

Sex	Age	Symptoms	Confirmed TB	Radiological Findings	Biologic	Indication
f	64	FUO	Yes	Miliary TB	Infliximab	Erythema Nodosum
f	61	NS, Weakness, Dyspnoea	No	Cavernous infilt. left	Infliximab	Crohn's disease
f	53	NS	Yes	Nodular densifications	Infliximab	Rheumatoid arthritis
f	69	FUO, NS, Cough	Yes	Stained infiltrations	Series of TNF-α Inh.	Rheumatoid arthritis
m	49	FUO	Yes	Pleural effusion left	Adalimumab	Rheumatoid arthritis
f	24	NS, Weakness	Yes	Miliary TB	Infliximab	Behcet's disease
f	28	Pulmonary Infection	Yes	Confluent densifications	Adalimumab	Behcet's disease
f	59	FUO, Cough	Yes	Upper lobe infiltrations	Certolizumab	Rheumatoid arthritis

TNF-α inhibitors-was to characterize screening failures and risk factors for de novo infections and to analyze the clinical manifestations of active tuberculosis in this cohort. The Otto-Wagner Hospital in Vienna is specialized in tuberculosis infections. Yearly around 150 patients are admitted. We identified eight patients with active tuberculosis during TNF-α inhibitor therapy by retrospective review of the clinical charts. The indication for treatment was rheumatoid arthritis in 4, Behcet's disease in 2, Erythema nodosum in 1 and Crohn's disease in 1 patient. Four patients received infliximab, two adalimumab, one certolizumab and one a series of TNF-α inhibitors. The patient group consists of seven women and one man. The mean time to diagnosis of active infection was 35.75 days (min 1 d; max 75 d). The symptoms were FUO in four cases, night sweats in four cases, cough in two cases, weakness in two cases and dyspnoea in one case. Quantiferon test was performed and positive in five out of eight patients, PCR was positive in four out eight patients, Mycobacterium tuberculosis was identified by culture in seven out of eight cases (bronchial lavage samples, sputum, thoracentesis and lung biopsy). Active tuberculosis infection occurs during therapy with TNF-α inhibitors although patients and treating physicians are aware of the risks and potential fatal sequels. Our cases show that not only screening failures are possible, but also follow-up of tuberculosis infection status is necessary, to diagnose tuberculosis reactivation and de novo infections in a timely manner. Furthermore clinical symptoms and manifestations differ from classical tuberculosis infections making diagnosis more difficult.

Figure 1

Figure 2

P.1.8.002 (B)

Resurgence of CNS tuberculosis among Bahraini children. Do we need to reintroduce universal BCG vaccination?

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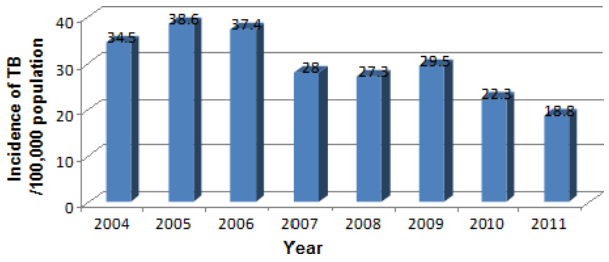
INTRODUCTION Central nervous system tuberculosis (CNS TB) is the most serious form of TB; accounting for 5% of EPTB (extrapulmonary TB) cases worldwide. Kingdom of Bahrain is considered as low endimicity area for tuberculosis (incidence rate 18.8/100 000 population in 2011). Childhood BCG vaccine which has been a part of TB control strategy in the kingdom in the past has been stopped since 2003 and replaced by targeted BCG vaccine to newborn of immigrants who came from communities where there is a high incidence of TB. During our clinical practice we have been observing reemergence of CNS TB among pediatric age group.

MATERIALS AND METHOD Retrospective study of all registered cases of CNS TB in Bahrain from 2004 to 2011.

RESULT The Incidence of TB in Bahrain is decreasing during the study period (Graph 1) but the percentage of CNS TB from the total reported extra pulmonary tuberculosis was on the trend of increasing during the same period (4.3% in 2004 to 6.3% in 2011) Table 1. The total number of registered CNS TB in the kingdom during the study period was 43 cases. The median age

was 33.5 years (range 1–66). The majority were male and Non-Bahrainis (79.07%) which is expected with the high influx of expatriate young male workforce in the kingdom who came originally from high endemic area for TB. The proportion of Pediatric cases (<15 years old) with CNS TB is increasing over the study period (Table 2). CNS TB among pediatric age group found only among Bahraini population, while NonBahrainis constitute the majority of patients among most other age groups (Graph 2). CONCLUSION CNS TB is reemerging among Bahraini children <15 years of age. This highlights the importance of revising the public health policy for TB control program and BCG vaccination in the kingdom.

Graph 1 Incidence rate of all TB cases in Kingdome of Bahrain (2004–2011).



Graph 2 Age group of Patient with cerebral tuberculosis stratified by nationality.

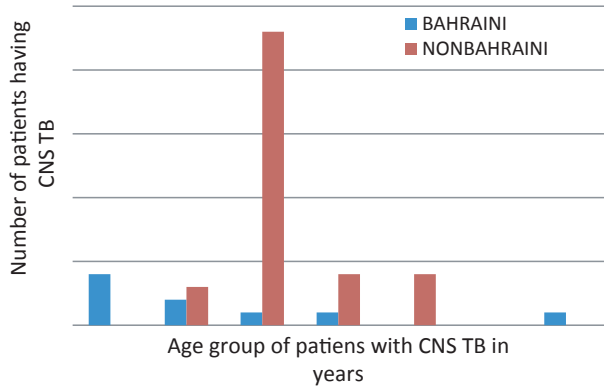


Table 1 Number & Percentage of CNS TB in Bahrain (2004–2011)

Year	Population	Number of TB cases	Number of EPTB	Number of CNS TB	% of CNS TB of EPTB	% of CNS TB of TB
2004	707 160	244	117	5	4.30%	2%
2005	724 645	280	109	0	0%	0%
2006	742 562	278	107	4	3.70%	1.40%
2007	1 039 297	291	111	6	5.40%	2.10%
2008	1 106 509	302	101	5	4.90%	1.70%
2009	1 107 000	327	118	12	10%	4%
2010	1 106 509	247	98	5	5.10%	2.%
2011	1 234 571	232	95	6	6.30%	2.60%

Table 2 Number of pediatric cases (<15 years old) with CNS TB in Bahrain (2004–2011)

Year	Number of cases <15 years of age with CNS TB
2004	0
2005	0
2006	0
2007	0
2008	0
2009	1
2010	1
2011	2

P.1.8.003 (B)

Knowledge and awareness of tuberculosis among general population in Chad

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In 2011, the prevalence of tuberculosis in Chad was 91 per 100 000 inhabitants and the case detection rate of smear positive pulmonary was still very low compared to the 70% target recommended by the WHO. Perceived stigma and lack of awareness could contribute to the late presentation and low detection rate of TB.
OBJECTIVE To assess the level of knowledge and determine the attitudes and practices of the general population regarding tuberculosis.
METHOD We conducted a cross-sectional study in urban areas. The selection of individuals and households was made in a random manner. It was done from 9 to 24 August 2012 in the health district of South N'Djamena. Data collection was conducted from door to door using a questionnaire.
RESULTS One hundred and forty-seven healthy subjects (97 males, 50 females), with a 32 years median age (range: 18–78) were included. Nearly all (96%) knew that TB was a pulmonary disease and could be contagious. Only 27.2% of them knew that air transmission was the predominant route. More than a half (60%) had some misconceptions about the possible modes of transmission. Participants quoted loss of appetite, haemoptysis, cough, as basic symptoms of TB. Only 30% of people knew the correct ways to prevent TB. Stigma toward TB was reported by 30% of people. One out of two subjects did not know that TB treatment was free of charge. Young male (under 35 years) and educated individuals were more likely to be aware about TB. Radio (48%) was the main source of information about TB.
CONCLUSION Our study showed that level of knowledge about TB is not satisfactory in Chad. TB control programs in Chad should educate general population, particularly female and non educated individuals.

Table 1 Preliminary results of the MSF TB screening protocol (1st August 2012 – 30th April 2013)

Center	Total new inmates	Total TB questionnaires (% among new inmates)	Positive TB questionnaires (% among new inmates)	Second evaluation (% among positive questionnaires)	TB (% among new inmates)
Milano	547	541 (98.9)	24 (4.4)	8 (33.3)	1 (0.2)
Roma	889	880 (99.0)	19 (2.1)	11 (57.9)	1 (0.1)
Trapani	416	385 (92.5)	11 (2.6)	4 (36.4)	2 (0.5)
Caltanissetta	79	78 (98.7)	0 (0)	0 (0)	0 (0)
Total	1931	1884 (97.6)	54 (2.8)	23 (42.6)	4 (0.2)

Table 2 Clinical characteristics of active TB cases (1st August 2012 – 30th April 2013)

	Sex	Age (years)	Nationality	Localization	Sputum Microscopy	Treatment compliance
Milano	M	38	Morocco	Pulmonary	Negative*	Good
Roma	M	42	Algeria	Pulmonary	Negative	Bad†
Trapani	M	18§	Morocco	Pulmonary	Negative	Bad†
Trapani	M	33	Ghana	Pulmonary	Not done	Not evaluable‡

*Diagnosed in prison (2012): in continuation phase treatment (5th month), released from the CIE for medical reasons.
†Escaped from Infectious Diseases Unit after 2 days of hospitalization.
‡Diagnosed in Palermo Infectious Diseases Unit (2012) with voluntary treatment interruption: re-started initial phase treatment inside the CIE.
§Just landed in Lampedusa island: reported 1 year of antibiotic treatment in Morocco for an unspecified lung disease.

P.1.8.004 (B)

Tuberculosis (TB) active case finding among irregular migrants held in closed centers of identification and expulsion (CIEs): preliminary analysis of Medecins Sans Frontieres intervention in Italy

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INTRODUCTION CIEs hold irregular migrants waiting to be expulsed from Italy. CIEs are managed by the Italian Minister of Interior but primary health care depends on private companies. Italian guidelines promote early TB diagnosis for migrants but active case finding procedures inside the CIEs are not available, so far. MSF's intervention set up a pilot programme of TB active case finding in 4 among 13 Italian CIEs.

MATERIALS AND METHODS MSF provided TB training for local staff, administered TB screening questionnaires to all inmates at admission in collaboration with local health staff and facilitated referrals to TB centers. TB questionnaires consisted of verbal screening on symptoms suggestive of active TB, previous history of TB or previous contact with a TB case.

RESULTS From August 2012 to April 2013, 1931 migrants were enrolled, 54 (2.8%) had positive questionnaires: the majority were men but HIV co-infected transsexuals had the highest risk of having a positive questionnaire due to previous TB. Most frequent answers were: previous history of TB (35%) and chronic cough (33%). Twenty-three (42.6%) were referred to TB centers. Reasons for not being referred were (in order): CIEs operational limitations, physician decision and host's refusal. Active TB was diagnosed in four individuals (0.2% among screened) (see Tables 1 and 2).

CONCLUSIONS These results confirm the higher incidence of active TB among irregular migrants in closed centres compared to general population living in Italy. The overall yield of this intervention is in the range reported for other migrant TB screening programmes in open contexts. Referral outside the CIEs was not optimal, mainly because of CIEs operational limitations: since a high number of positive questionnaires were not referred, in order to ensure universal access to secondary health care, more effort must be done by CIEs staff towards the completion of the diagnostic workup.

P.1.8.005 (B)

High prevalence of clustered tuberculosis cases in the Peruvian community in Florence, 2001–2010

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INTRODUCTION Peruvians are the largest Latin American community in Italy with a sizable group of 7834 subjects living in the city of Florence. Tuberculosis (TB) is a leading cause of morbility for Peruvian migrants in Florence where they account for more than 15% of TB cases in recent years.

MATERIALS AND METHODS Relevant features of TB cases notified in Peruvian citizens resident in Florence in the period 2001–2010 were retrospectively collected. Genotyping analysis

Table 1 Clinical, microbiological and epidemiological features of tuberculosis cases notified in Peruvian citizens resident in the Health District of Florence, Italy, in the period 2001–2010

	All cases (138)	Cases with genotyping available (87)	Clustered cases (39)	Non clustered cases (48)	OR	*P* value (clustered versus non clustered)
Female sex	79/138 (57.2%)	55/87 (63.9%)	22/39 (56.4%)	33/48 (68.7%)	0.59 (0.22–1.55)	0.23
Age ≤15 years	15/138 (10.9%)	9/87(10.3%)	6/39 (15.3%)	3/48 (6.2%)	2.73 (0.55–15.03	0.15
Age 16–50 years	120/138 (86.9%)	77/87 (88.5%)	33/39 (84.6%)	44/48 (91.7%)	0.50 (0.11–2.23)	0.24
Age >50 years	3/138 (2.2%)	1/87 (1.1%)	0/39 (0%)	1/48 (2.1%)	0 (0–22.75)	0.55
Diagnosis of tuberculosis within 5 years from arrival in Italy	81/116 (69.8%)	52/83 (62.6%)	20/32 (62.5%)	32/48 (66.7%)	0.83 (0.3–2.35)	0.70
Unemployed	34/115 (29.6%)	22/87 (25.3%)	8/38 (20.5%)	17/48 (35.4%)	0.49 (0.16–1.43)	0.14
Diagnostic delay >60 days	46/113 (40.7%)	32/72 (44.4%)	12/32 (37.5%)	20/40 (50%)	0.60 (0.21–1.71)	0.28
Pulmonary localization only	106/136 (77.9%)	68/86 (79.1%)	32/38 (84.2%)	36/48 (75%)	1.78 (0.54–6.09)	0.29
Pulmonary and extrapolmonary localization	8/136 (5.9%)	5/86 (5.8%)	3/38 (7.9%)	2/48 (4.2%)	1.97 (0.25–18.02)	0.38
Extrapulmonary localization only	20 (14.7%)	12 (13.9%)	3/38 (7.9%)	9/48 (18.7%)	0.37 (0.07–1.68)	0.14
Disseminated tuberculosis	2 (1.5%)	1/86(1.2%)	0/38 (0%)	0/48 (0%)		
Smear positive tuberculosis among subjects with pulmonary localization	60/114 (52.6%)	43/73 (58.9%)	19/35 (54.3%)	24/38 (63.1%)	0.69 (0.24–1.96)	0.44
HIV prevalence	2/73 (2.7%)	1/47 (2.1%)	0/25 (0%)	1/27 (3.7%)	0 (0.19–31)	0.51
Microbiologically confirmed cases	119 (86.2%)	87/87 (100%)	39/39 (100%)	48/48 (100%)		
Mycobacterium tuberculosis strain sensitive to H, R, P, E	93/113 (83.3%)	75/84 (89.3%)	37/38 (97.4%)	38/46 (82.6%)	7.79 (0.90–174.27)	**0.02**
MDR strains	9/113 (8%)	2/84 (2.4%)	0/39 (0%)	2/46 (4.3%)	0 (0–4.88)	0.28

H, Isoniazid; R, Rifampin; P, Pirazinamide; E, Ethambutol; MDR, Multi Drug Resistant.

of available Mycobacterium tuberculosis (Mt) strains isolated was performed using Mycobacterial Interspersed Repetitive Units (MIRU) and Spoligotyping analysis. A cluster was defined as two or more patients with clonally related strains. Clustered patients were assumed to belong to a chain of recent transmission; patients whose Mt isolates displayed unique patterns were assumed to have reactivated TB.

Results Characteristics of the 138 TB cases retrieved are summarized in Table 1. The notification rate gradually decreased from 484 cases per 100 000 Peruvian residents in 2001 to 362 in 2010. Fourteen (93.3%) cases diagnosed in subjects aged 15 or less were diagnosed after 2005. Genotyping was performed in 87 Mt strains showing that 48 cases (55.2%) were non clustered while 39 (44.8%) belonged to 12 clusters (involving 2–6 subjects each).

Conclusions The study confirms that TB is a concerning problem for the Peruvian Community in Florence. The notification rate is threefold if compared to those of Peruvians living Italy or in Peru (381 vs. 117 vs. 113 in 2009). An elevated proportion of cases in this population belongs to a chain of recent transmission and could be prevented by implementing adequate strategies such as early diagnosis of active TB cases and contact tracing procedures. This work has been supported by the EC within the 7th Framework Programme under the COHEMI project – grant agreement no. FP7-GA-261495.

P.1.8.006 (B)

A review of paediatric tuberculosis in Denmark: 10 year trend, 2000–2009

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Introduction Paediatric tuberculosis (TB) is a key indicator for recent transmission and presents a reservoir for the disease. We describe trends in epidemiology, microbiological characteristics and treatment outcome in Denmark between 2000 and 2009.

Material and Methods Data was retrieved from the national TB surveillance system and the International Reference Laboratory of Mycobacteriology.
Results In total, 323 TB cases were reported in children below the age of 15 years, accounting for 7.6% of all notified cases in Denmark. The overall incidence rate of childhood TB declined from 4.1 per 100 000 to 1.9 per 100 000 in the study period. Immigrant children comprised 79.6% of all cases, with the highest incidence rate of 94.1 per 100 000 children in 2001. In contrast to immigrant children, the majority of Danish children were younger than 5 years and with known exposure to TB. Pulmonary TB was the commonest presentation. Only half of the cases were culture confirmed.
Conclusions We observed an overall decreasing trend in the children to adult notification ratio, but a slight increase in the ratio when calculated specifically for ethnic Danes. Childhood TB needs continuous attention with special focus on risk groups. Emphasis on improving early TB case detection, contact tracing and further implementation of preventive treatment is necessary.

P.1.8.007 (B)

Safety, tolerability and 14-day antimycobacterial activity of the novel antituberculosis compound SQ109

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Introduction New, more effective anti-TB drugs are needed to shorten the treatment of drug-sensitive TB and overcome drug resistance. SQ109 was developed by Sequella Inc. from the diamine pharmacophore of ethambutol. It strongly synergizes with rifampicin (RIF) and novel anti-TB drug candidates. Intracellular activity has been confirmed in human whole blood culture. In phase 1 studies SQ109 was well tolerated and safe. We report the results of the first SQ109 administration to TB patients.
Materials and Methods We randomized six groups of 15 patients to receive 75 mg SQ109, 150 mg SQ109, 300 mg SQ109, 150 mg SQ109 + 10 mg/kg RIF, 300 mg SQ109 + 10 mg/kg RIF, or 10 mg/kg RIF, daily for 14 days. Patients were hospitalized for safety and tolerability monitoring. From daily overnight sputum cultures we measured the 14-day early bactericidal activity (EBA) by the daily fall of colony forming units on solid agar (logCFU/ml sputum ±SD), and the daily increase of time to culture positivity in liquid media (TTP in hours ±SD).
Results RIF-containing regimens showed a linear decline in log10 sputum mycobacterial load over time (RIF alone: −0.093/day; 95% CI; −0.126 to −0.059), consistent with published data. SQ109 had no apparent effect in monotherapy or when added to RIF. SQ109 was well tolerated, the most frequent AEs were gastrointestinal complaints. Pharmacokinetics were dose-related but not dose-proportional. A decrease in SQ109 exposure with RIF-coadministration was overcome at the 300 mg dose.
Conclusions SQ109 was safe and well tolerated. The lack of activity in the first 2 weeks of treatment is not unexpected and can be explained with slow intracellular accumulation of the drug. Since 14-day EBA studies may not accurately predict the effects of the drug during later stages of treatment, the efficacy of SQ109 300 mg added to standard antituberculosis agents over 12 weeks is currently evaluated in a Phase II study by the PanACEA consortium.

P.1.8.008 (B)

Extra-pulmonary tuberculosis and its comparison with pulmonary tuberculosis in Iran

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Background *Mycobacterium tuberculosis* usually infects the lungs but Organs other than the lungs may also be involved. This study is an analysis of the situation of extra-pulmonary tuberculosis in the central province of Iran.
Methods and Materials In this cross-sectional study, the information in the registration software of tuberculosis in Health centers collected and for analyzing of data statistical software SPSS ver.16 was used.
Results In the survey a total of 1787 TB patients were identified, of which 24.2% were diagnosed with extra-pulmonary tuberculosis and %1.9 of patient with extra-pulmonary TB were associated with HIV infection. Female to male sex ratio is equal to 1.3. The 82.5% of the patients were Iranian citizen and mean age of patients were 43.3 years. Tuberculosis of the lymph nodes, skletal and pleural tuberculosis had the highest prevalence. Also extra-pulmonary tuberculosis in females, age15–55 was more and the diagnosis delay was more in extra-pulmonary tuberculosis and relapse was more in pulmonary tuberculosis.
Conclusions Because of more diagnosis delay in extra-pulmonary tuberculosis it is necessary to train physicians and other healthcare workers in the field of extra-pulmonary TB diagnosis and more planning to do about learning of it.

P.1.8.009 (B)

Comparison of the genetic convergence of mycobacterium strains by three RFLP based methods

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Background and Objective The occurrence of molecular techniques in the epidemiology of tuberculosis provided an opportunity to use effective markers for the tracing of the phenomenon of transmission of the disease.The purpose of this study was to compare the *Mycobacterium tuberculosis* genetic patterns by three methods of RFLP technique.
Materials and Methods In a cross sectional and prospective study 95 strains of M.tuberculosis isolated were selected for DNA fingerprinting. Extraction of DNA from Mycobacterium strains and DNA fingerprinting with IS-6110, PGRS and DR probe were performed by standard protocols of WHO.
Results In overall, the diversity by RFLP of 95 tuberculosis patients, were 48, 50 and 45 on the basis of IS6110, PGRS and

DR patterns respectively. Twenty of these patterns (21.1%) with IS6110-RFLP, 22 (23.2%) with PGRS-RFLP and seventeen (17.9%) with DR-RFLP occurred with unique RFLP patterns whereas, the remaining 28 patterns were shared. Tuberculosis patients were more likely to be in a cluster if they were younger than 45 years, were new cases, degree of sputum smear were ≥2+ and were close contact.
CONCLUSION Our study demonstrated that IS6110-RFLP, PGRS- RFLP and DR- RFLP genotyping identified roughly similar proportions of clustered (secondary) cases as well as the same risk factors for clustering.

P.1.8.010 (B)

Interferon-gamma-induced protein 10 as a diagnostic marker of pulmonary tuberculosis after treatment initiation

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INTODUCTION Pulmonary Tuberculosis (PTB) is a major public health problem worldwide. The gold standard for diagnosis is culture, but its clinical utility is limited because of the time required to obtain results. In resource-limited settings, PTB is usually confirmed by sputum smear microscopy. This method is insensitive and many patients are clinically diagnosed as having smear-negative PTB, but their diagnosis remains uncertain and they often have poor treatment adherence. Thus, a confirmatory test could be very valuable. IP-10 is a biomarker that can identify TB infection. A study in TB-infected mice demonstrated that IP-10 blood levels increased in the first week after TB-treatment initiation and decreased the following week. This increase was absent in TB-infected mice treated with placebo and in uninfected mice. There are no studies describing the pattern of IP-10 in humans during the first week of TB-treatment. This study describes the pattern of IP-10 blood concentration in adults with smear-positive and smear-negative PTB during the first 2 weeks of treatment, comparing their IP-10 concentrations with adults without TB.
METHODS This is a descriptive case-series of 40 adults with new PTB initiating anti-TB treatment from May to June 2013 in Kathmandu. Patients previously treated for TB or with a history of contact with multi-drug-resistant TB will be excluded. Patients will be classified as smear-positive/culture-positive, smear-negative/culture-positive and smear-negative/culture-negative. Blood samples will be collected before anti-TB therapy and on days 1, 3, 5, 7 and 14 after treatment initiation. IP-10 concentrations will be quantified using ELISA. A summary of the results will be presented at the conference.

P.1.8.011 (B)

Intra cavitary aspergilloma indicative of ulcerative caseous tuberculosis late discovery: about a case

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ABSTRACT
BACKGROUND AND RATIONALE Ulcerative caseous tuberculosis rife in endemic form in countries with low socio-economic level, some past unnoticed forms are discovered late in the course of a complication if hemoptysis is a frequent complication this form of tuberculosis, the recurrent character must guide the investigation in search of a transplant aspergillosis.
COMMENT The author reports the case of recurrent hemoptysis high abundance in a patient 34 years old health worker, non-smoking, HIV negative, no history of particular medical emergencies admitted in July 2012. After repeated blood transfusions, he was hospitalized in the Pneumophtisiology where clinical and laboratory investigations show an ulcerative caseous tuberculosis. A first-line TB treatment is instituted. But before the recurrent nature of this hemoptysis involving life-threatening, a CT scan is performed highlighting transplant aspergillosis associated. Surgery of pulmonary resection was performed in the management of pulmonary aspergilloma with uneventful.
CONCLUSION Ulcerative caseous tuberculosis is often revealed by hemoptysis in tuberculosis endemic areas where these forms are late discovery. In this context, to recurrent hemoptysis, the positivity of the sputum smear should not limit the realization of a CT scan which to diagnose intra cavitary aspergilloma associated.
KEYWORDS Aspergilloma intra cavitary, ulcerative caseous tuberculosis, hemoptysis

P.1.8.012 (B)

Latest results of ongoing evaluation of the loop-mediated isothermal amplification (LAMP) assay for the diagnosis of tuberculosis in University Teaching Hospital (UTH), Zambia

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INTRODUCTION Accurate, point-of-care TB diagnosis is necessary to treat patients successfully and reduce TB infections in communities of developing countries. The main objective of this study is to establish a new rapid diagnostic test based on LAMP (Loop-mediated isothermal amplification) for the detection and differentiation of Mycobacteria in Zambia. An in-house (Hokudai: Hokkaido University) LAMP is being evaluated for its sensitivity and specificity at the TB laboratory, UTH, Zambia.
DESIGN AND METHODS Sputa were collected at the chest clinic, UTH. One to three sputum samples with informed consent per suspect were used. Smear microscopy, decontamination, culture and identification tests were performed according to the standard. Two ÀμL samples treated with NALC-NaOH, or concentrated by centrifugation were used for LAMP assay. Comparison was made between LAMP results and direct smear microscopy results, MGIT culture results, and identification using Capilia TB.
RESULTS Comparing LAMP results to smear results, the sensitivity was 95% (46/48), and the specificity was 90.9% (60/66). However, comparing LAMP results to culture results, the sensitivity was 68.7% (46/67), and the specificity was 89.6% (60/67). The sensitivity compared with cultures was lower than expected. The reason for lower sensitivity is still under investigation, but concentration of NALC or quality of distilled water might be a problem as an inhibitor.
CONCLUSIONS To raise the sensitivity of LAMP, PURE LAMP (Eiken Chemical Co., Ltd., Tochigi, Japan) will be introduced as a DNA extraction procedure. For the next study, the results of direct smear, MGIT culture, Eiken LAMP, Hokudai LAMP, and GeneXpert will be compared.

1.10 Diarrhoeal diseases

P.1.10.001 (A)

Gastrointestinal infections in Ghanaian children and associated disease symptoms – a hospital based case–control study

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Diarrhoea is one of the leading causes for childhood mortality in sub-Saharan Africa. However, little is known about gastrointestinal (GI) infections and their associated symptoms to facilitate clinical diagnosis. Aim of this study was to describe the frequency of GI infections in children in Ghana and the association between infection and diarrhoea symptoms. In 2007/2008 stool samples were collected from children (<13 years) with diarrhoea (cases) and without diarrhoea (controls) admitted to a hospital in rural Ghana. The following organisms were tested using PCR: *Campylobacter jejuni*, *Cryptosporidium parvum*, *E. histolytica*, *Giardia lamblia*, Norovirus, Rotavirus, *Salmonella enterica*, *Shigella* spp./EIEC and *Yersinia* spp. A case-control study was conducted to describe associations between infections and diarrhoea. Age-stratification was applied and Mantel-Haenszel odds ratios (ORMH) with 95%-confidence intervals (CI) were calculated to adjust for age. Stool samples were taken from 1520 children (583 cases and 937 controls). Most frequent isolates were *Shigella* spp./EIEC ($N = 381$; 25.1%), *Campylobacter jejuni* ($N = 304$; 20.0%), and Norovirus ($N = 166$; 10.9%). *E. histolytica* and *Yersinia* spp. were not isolated. The age of the children differed strongly amongst infections. Rotavirus, Norovirus, and *Cryptosporidiun parvum* mainly affected children below 2 years, whereas *Campylobacter jejuni*, *Shigella* spp./EIEC, and *Salmonella enterica* primarily affected children above 2 years. In the stratified analysis age was proven to be a strong confounder. Rotavirus (ORMH 2.2; CI 1.3–3.7), *Cryptosporidium parvum* (ORMH 1.9; CI 1.1–3.2), and *Shigella* spp./EIEC (ORMH 1.7; CI 1.3–2.2) showed the strongest associations. Giardia lamblia was not associated with diarrhoea. There was no predominating organism associated with diarrhoea amongst the samples tested. However, considering our results along with information such as age and exposure history of the affected children would guide clinicians to set their diagnoses. Furthermore, the study revealed strong age confounding, which should be considered in such analyses.

1.10.1 Rotavirus

P.1.10.1.001 (A)

Modelling the costs of implementing the rotavirus vaccine into the national immunisation programme: the case of Malawi

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INTRODUCTION Rotavirus infections are responsible for approximately 453 000 child deaths annually, equalling 37% of all diarrhoeal deaths among children. Two licensed rotavirus vaccines could be life- and cost-saving in low-income countries where the disease burden is highest. The World Health Organization recommends that these vaccines are included in national immunisation programmes worldwide. Prior to vaccine introduction, it is crucial to estimate vaccine costs as well as system costs related to the implementation for budgetary purposes and to assess potentials for savings. The aim of our study was to model the total cost of implementing the rotavirus vaccine in Malawi and to examine the relative contribution of different cost components to the total cost.
MATERIAL AND METHODS The World Health Organizations guidelines for cost analyses of vaccine introduction into national immunisation programmes were employed to model the resource use and costs associated with rotavirus vaccine implementation in Malawi for a 5-year period (2012–2016). The analysis was undertaken from a governmental perspective. Scenario- and sensitivity analyses were performed.
RESULTS The total cost of vaccine implementation in Malawi amounted to US$ 5.8 per child in the birth cohort. Eighty-three percent of the total cost was attributed to vaccine purchase, whereas 17% was attributed to system costs. With GAVI Alliance financial support, the total cost was reduced to US$ 1.4 per child in the birth cohort, while vaccine purchases were reduced to constitute 29% of the total cost.
CONCLUSIONS The total cost of rotavirus vaccine implementation in Malawi is high compared to the governmental health budget per capita. Initially, GAVI support will cover the majority of the costs. However, once this support expires, Malawi could face significant financing challenges. This highlights the need for new global initiatives to facilitate vaccine implementation and to ensure sustainable financing in low-income countries.

1.10.2 Cryptosporidium a public health concern – recent advances in our understanding of the epidemiology

P.1.10.2.001 (A)

Zoonotic transmission of *Cryptosporidium felis* in a household

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Most human cases of cryptosporidiosis are caused by *Cryptosporidium hominis*, which mainly infects humans, and *C. parvum*, a species with a confirmed zoonotic potential. The use of molecular methods has revealed that several other species, like *C. canis* and *C. felis* (naturally infecting dogs and cats respectively) also can cause human infection. However, recent findings suggest that most of these infections, which mainly occur in developing countries, are caused by anthroponotic rather than zoonotic transmission. Thus the role of companion animals in human cryptosporidiosis is not clear. Here we have investigated a case of suspected zoonotic transmission of cryptosporidiosis in Sweden. Stool samples from a cat and a women, both suffering from diarrhoea and living in the same household, were analysed using microscopy after modified Ziehl-Neelsen staining and PCR-RFLP analysis of the COWP and SSU rRNA genes followed by sequencing. Microscopy of stool samples from both individuals showed the presence of *Cryptosporidium* spp. oocysts, while molecular analysis confirmed the presence of *C. felis*. We report the first confirmed case of cryptosporidiosis caused by transmis-

sion of *C. felis* between a cat and a human living in the same household. Due to the fact that the cat developed symptoms before the patient, and that human cryptosporidiosis caused by *C. felis* is a rare occurrence in Sweden, we consider it probable that the cat was the initial source of infection, and not the opposite. Our results show that Cryptosporidium infection can be achieved from a companion animal and that molecular analysis is necessary for confirmation.

P.1.10.2.002 (A)

Microsporidia is clinical significance and newer methods for its detection in routine parasitology laboratory

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BACKGROUND Diarrhea is the second leading cause of morbidity in HIV seropositive patients. Globally the prevalence of Microsporidia has been reported variably from 5 to 40%.The vast variation is largely due to difference in the diagnostic technique employed.
AIM To determine (i) Prevalence of Microsporidia and its comparison to other parasites as a cause of diarrhea in HIV positive patients. (ii) Comparison of Trichrome based staining techniques in the diagnosis of Microsporidia. (iii) Clinical features of microsporidial infection.
MATERIALS AND METHODS Prospective 1 year was carried out in a 1800 bed tertiary care teaching hospital. Hundred and eight patients were enrolled for this study. Written informed consent of all patients prior to enrollment in the study was taken.
RESULT The correlation of Microsporidial infection with CD4 counts of the patients was done. The prevalence of parasitic diarrheas among HIV positive patients was done. Common clinical findings in patients with Micro sporidial diarrheas were weight loss, anorexia and abdominal pain .Modified ZN staining, Ryan Blue and Weber Green were compared for the detection of Microsporidia.
CONCLUSION Microsporidia are an important cause of parasitic diarrhea and significant weight loss in severely immunocompromised patients. Detection of Microsporidia in stool is missed completely by routine lab investigation. Trichrome staining is an inexpensive, non invasive, easy and rapid method for detection of Microsporidia in stool. Both Ryan Blue and Weber Green had similar detection rates. However Ryan Blue had an advantage in terms of ease of detection of Microsporidia and no confusion with yeasts and other artifacts. So, all routine labs performing stool examination should incorporate modification of Trichrome staining as a part of routine protocol for diagnosis of Microsporidia.

P.1.10.2.003 (A)

Distribution of intestinal parasites prevalence in children according to the distribution of the patients gender and parasite species in Eskisehir Osmangazi University Hospital

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OBJECTIVES Intestinal parasite infection is still an important public health problem. In this study, patients (out patients and in-patients) with various gastro intestinal system complaints presenting at pediatric clinics of the Osmangazi University Medical Faculty, from January 2004-December 2012 were investigated retrospectively for intestinal parasites.
METHODS Parasitological examinations were done with native-lugol, cellophane-tape methods and trichrome staining in ambiguous cases. Also modified Erlich-Ziehl-Nielsen staining was used to identify Cryptosporidium spp.
RESULTS In a total of 16 224 cases examined 2.3% were found infected with at least one or more parasite, without difference between genders (including nonpathogenic protozoa). In our area predominant parasites were Blastocystis hominis 23% and Entamoeba histolytica/dispar and other group amoebas. The distribution of identified parasites was as follows; Giardia intestinalis7%, and *Cryptosporidium parvum* 4.5%. Since the cellophane type method was only used in a few cases. The ratio of *Enterobius vermicularis* was found to be 2.3% Taenia saginata 0.8% and *Strongyloides stercoralis* 0.4%. In comparison to a previous 10 year retrospective study which was performed in our hospital, we detected an important decrease in prevalence of parasites. But the presence of intestinal parasites is still an important problem.
CONCLUSION This study re-emphasizes the fact that intestinal parasitic infection is still an important public health problem. Interventions including health education on personal hygiene to the student sand to the parents, especially to mothers are required.

P.1.10.2.004 (A)

Detection and molecular characterization of human isolates of Giardia lamblia from the United Arab Emirates

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Giardia lamblia, the intestinal protozoan causing giardiasis, infects about 200 million individuals annually worldwide. The spectrum of clinical manifestations ranges from an asymptomatic infection to a severe diarrhea. There are seven different assemblages of *G. lamblia*, where two (A and B) assemblages have been associated with humans, while the others might have a zoonotic potential. The aim of the study was to accurately identify *G. lamblia* in DNA isolates extracted from human stool samples in order to gain information on its accurate prevalence and also to assess its genotypic composition in Sharjah, UAE. One hundred and eleven healthy expatriates residing in Sharjah and attending the Sharjah Municipality Clinic have been screened for *G. lamblia* using nested PCR amplification of the Small Subunit rRNA (SSU-rRNA) gene. Samples identified as positive for *G. lamblia* were genotyped using a nested PCR amplifying the triosephosphate isomerase (tpi) gene to differentiate between the two human assemblages (A and B). 63/111 (57%) were identified as positive for the SSU-rRNA gene. However, when genotyped for the tpi gene 28.6% were assemblage A, 27% assemblage B and 7.9% were mixed infections (A and B assemblages). Interestingly, 37% of the SSU-rRNA positive samples were neither assemblage A nor B. To our knowledge, this study is the first one to examine the degree of infection with this important parasite in the country and to accurately determine the infection rate and its genotypic composition. It also raises questions about the possible zoonotic potential of the organism as well as the transmission dynamics between expatriates and natives of the UAE.

P.1.10.2.005 (A)
Occurrence of Giardia duodenalis genotypes in sheep and goats in Tehran, Iran

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Introduction Giardia duodenalis is one of the most common and important gastrointestinal protozoan parasites in humans and animals, especially in developing countries. Based on genetic differences, G. *duodenalis* isolates classified into several genotypes, host-specific and potentially zoonotic genotypes. This study was performed to determine the occurrence of Giardia genotypes in sheep and goats in Tehran slaughterhouses.
Material and Methods Two hundred forty faecal samples were collected from the rectum of sheep and goats in Tehran slaughterhouses, during April to November 2011. The presence of G. *duodenalis* cysts were microscopically examined after sucrose gradient purification. For the molecular identification of Giardia, a semi-nested PCR protocol was used to amplify a 432 bp fragment of the glutamate dehydrogenase gene (gdh). The amplified fragments were subjected to restriction analyses and direct sequencing of purified DNA gel bands for the genotyping assays.
Results Microscopic examination found 56 of 240 (23.3%) sheep and goats infected with Giardia. Based on molecular identification, the frequency of Giardia was 49.2% (118/240). Restriction analyses of the gdh fragments showed 44 (37.3%) assemblage E, 25 (21.2%) assemblage AI and 14 (11.9%) assemblage AII. Eighty one (68.6%) isolates were mixed genotypes (assemblage E with BIII, AI or AII). The potential zoonotic assemblages of G. *duodenalis* were found in 64.4% (76/118) of sheep and goats.
Conclusion The molecular identification revealed Giardia as a common parasite in sheep and goats in Tehran slaughterhouses. The presence of zoonotic assemblages (AI, AII, and BIII) in sheep and goats suggests their possible role as reservoirs of human giardiasis.

P.1.10.2.006 (A)
An investigation of Giardiasis and Cryptosporidiosis in Malawi and Cambodia

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The parasites, *Giardia* sp. and *Cryptosporidium* sp. infect both humans and animals and are considered to have the potential for zoonotic transmission. Giardiasis, caused by *Giardia duodenalis*, is one of the most common intestinal protozoal infections reported worldwide particular in children. At least seven morphologically identical genetic assemblages (A to G) of G. duodenalis have been categorised. Cryptosporidium is a protozoan parasite and emerging pathogen that has become recognised as a significant cause of protracted diarrhoea in both immunocompetent and immunocompromised individuals worldwide. At least seven *Cryptosporidium* species have been reported to infect humans. The two species of greater significance in terms of public health are *C. parvum* and *C. hominis*. Multilocus sequence typing (MLST) is a useful approach for detecting the presence of genotypes of these parasites. Genotyping of human isolates of both parasites from the Malawi and Cambodia can provide crucial information about transmission routes and epidemiological differences between Giardia assemblages and Cryptosporidium species. The purpose of this study was to determine the prevalence and range of *G. duodenalis* assemblages and *Cryptosporidium* species infecting children from Malawi and Cambodia and investigate the use of Gas Chromatography and Mass Spectrometry (GCMS) as a novel method for studying the volatile organic compounds in faeces of patients with *Giardia* and *Cryptosporidium* infection. Faecal samples were collected from children under 5 years of age, living in diverse geographical regions in Malawi and Cambodia. Parasite isolates were typed by using a combination of both PCR and restriction fragment length polymorphism (RFLP) and/or sequencing of SSU-rRNA, Ì²-giardin, tpi and gdh genes. Initial results confirmed the occurrence of mixed infections with assemblage A and B genotypes, and also mixed infection with *C. hominis* and *C. parvum*.

1.11 Yellow fever

P.1.11.002 (B)
Yellow fever: threat to Europe

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The last epidemic of yellow fever in Europe was in 1861 in St. Nazaire, a port city in France. But in the last 10 years, cases of yellow fever have been imported from Africa or South America into Belgium, the Netherlands and Switzerland. These countries are north of the range of the urban vector, *Aedes aegypti*, so there was no epidemic risk from that mosquito. But another urban vector, *Ae. albopictus*, has become established in those 3 and 12 more European countries. In 2007, a single individual infected with chikungunya virus arrived from India in northern Italy, outside the range of *Ae. aegypti*. He sparked an epidemic vectored by *Ae. albopictus*. Europe is now vulnerable to importations of yellow fever. Non-stop airline flights between endemic areas of South America and Africa can bring passengers incubating the virus to major European cities within 24 h, where they can fall ill and infect local mosquitoes. There have recently been urban outbreaks of yellow fever in the capital cities of Asuncion, Paraguay and Abidjan, Cote d'Ivoire, which have international airports serving intercontinental flights. Countries in Europe where those and similar flights land should be on the lookout for cases. Unless the physician seeing a case elicits a travel history from an endemic country, yellow fever would never be suspected. ProMED <www.promedmail.org> was searched for up-to-date news of yellow fever outbreaks in the endemic zones, and the spread of the vector mosquito species. The results will be presented. Insecticide spraying of aircraft cabins is not sufficient alone. The conclusion is that the present risk of a yellow fever epidemic in Europe is real, and contingency planning is urgently needed.

1.12 Food safety

P.1.12.001 (B)

Microbiological quality of oysters expended in Puebla City

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Seafood is involved in large cases of foodborne diseases each year around the world. It is estimated that just in the U.S.A, there are around 80 million of foodborne diseases per year. Our main goal was to analyze the microbiological quality of oysters that usually people consume without cooking. For this study 25 different samples were analyzed from different seafood establishments around Puebla City; 12 from restaurants and 13 mexican markets. The place selection was done by a random sampling using random number method and for the microbiological quality criteria it was used the Official Mexican Norm MX-242-SSA1-2009 that include the analysis of fecal coliforms, *Staphylococcus aureus*, *Salmonella* and *Vibrio cholerae* and Official Mexican Norm MX032-SSA1-1993 for mesophilic. 41.6% of restaurants and 38.5% of Mexican markets on mosophilic counts were out of norm. For fecal coliforms was found that all establishments were out of norm because the count exceeding. *Salmonell* sp., was found in 8% of samples from restaurants and 15.38 in Mexican markets. The S. aureus counts were high in 41% in restaurants and 61% in Mexican restaurants. These results indicate that the sea food expended in almost 50% of restaurants or Mexican markets poor quality and is important to do preventive programs mainly for tourist people to reduce to possible gastroenteritis problems.

P.1.12.002 (B)

Microbiological quality of Ceviche expended in Puebla City

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Sea food is involved in large cases of foodborne diseases each year around the world (80 million of foodborne diseases per year in USA). The main goal of this study was to evaluate the bacterial quality of one of the most famous dishes, 'ceviche'. For this study there were collected 25 samples from different seafood establishments around Puebla City, eight from classic Mexican markets and 17 from restaurants. The place selection was done by a random sampling using random number method and for the microbiological quality criteria it was used the Official Mexican NORM-MX242. In general, the 37% of the establishments in Mexican markets and 53% of restaurants had high mesophilic counts, being out of the official norm limits. 8% of these were out from the limits of the norm for fecal coliforms, indicating a direct human feces contamination. The *Staphylococcus aureus* counts were considered out the norm in 66% of the markets and in 24% of the restaurants. Regarding *Salmonella* results there were isolated four strains, two from one market sample (*S. choleraesuis* arizonae y *Salmonella* spp) and the rest from two different restaurants (*S. paratyphi* A y *S. choleraesuis* spp). It was found that just in one restaurant all counts for all criteria were out of the permitted limits. The Mexican markets had in general less sanitary controls to manage food and is congruent with the results founded according mesophilic, total and fecal coliforms and S. aureus too, however for Salmonella species, in two restaurants samples were isolated and one from market, indicating a people bad handling, probably having a healthy carrier cooking. Fortunately, *V. cholerae* was not found, but the lemon juice is not as good to eliminate all potential pathogens including *Salmonella*.

P.1.12.003 (B)

High antimicrobial-resistance levels of *Escherichia coli* isolated from meat of several markets in Lima, Peru

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Introduction *Escherichia coli* may contaminate foods. Thus alimentary *E. coli* outbreaks occur worldwide and have been linked to many food vehicles, including meat. In this way, food act as a bacteria transmission source and as a way of dissemination of antimicrobial resistance. Te aim of this study was to determine the antibiotic resistance levels of *E. coli* strains isolated of different types of meat marketed in Lima, Peru.
Material and Mthods Six markets of different areas of Lima were included in this survey. In total were collected 138 samples belonging to three different types of meat, Poultry (64), Swine (30) and Beef (44). Microorganisms were isolated by culturing in selective media. Suspicious *E. coli* strains were confirmed by amplification of the uidA gene. The susceptibility to Ampicilin, Tetracycline, Nalidixic acid, Ciprofloxacin, Azithromycin, Amoxicilin plus Clavulanic Acid, Chloramphenicol, Imipenem, Furazolidone and Rifampicin was established by disk diffusion.
Results From the collected 138 samples, 191 *E. coli* were recovered, from these 113, 44 and 34 were isolated from poultry, swine and beef, respectively. The microorganisms showed high levels of antimicrobial resistance to Rifampicin (96.86%), Ampicillin (74.35%), Tetracycline (75.92%), Nalidixic acid (57.07%), Ciprofloxacin (39.27%), Chloramphenicol (37.70%) Furazolidone (11.52%), Amoxicilin plus Clavulanic (6.28%) and Azithromycin (5.24%) while no resistance to Imipenem was observed. In general, samples from poultry showed higher levels of antibiotic resistance than those of swine or beef.
Conclusions These strains exhibit an important antimicrobial resistance levels. In Peru, *E. coli* isolates from meat is an important reservoir of resistant genes, which may be either transferred to commensal microorganisms of intestinal microbiota and to pathogenic microorganisms.

P.1.12.004 (B)

Human case of Fasciolosis in Serbia

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Introduction The number of humans infected by *Fasciola hepatica* is increasing worldwide. Humans can become accidental hosts by ingesting drinking water or plants contaminated with metacercariae. We report a case of acute HF of uncertain origin and source of infection in which this diagnosis has been established after serious diagnostic concerns.
Material and Methods A chronological record of the clinical observations of the patient's condition, diagnostic and therapeutic procedures and test results.
Results A 68-year-old Serbian woman was admitted at the Clinic for Infectious and Tropical Diseases in Belgrade due to

intermittent fiver, right upper quadrant abdominal pain, undefined lesions in liver, high eosinophilia (43.7%) and high activity of alkaline phosphatase (174 U/l). Our patient had a 3-months history of disease progression without a reliable diagnosis. We performed detailed diagnostic re-evaluation. Abdominal US showed a homogeneous liver of normal size, with no visible focal changes. Two enlarged periportal hilar lymph nodes with hypoechoic appearance were detected. Abdominal CT showed hypodense clustered lesions in a periphery of the right lobe of the liver and enlarged retroperitoneal lymph nodes. Based on clinical picture, abdominal sonography and CT findings, haematological and biochemical parameters and repeated investigation of liver biopsy she was appointed as suspected to the acute fasciolosis. Stool and duodenal aspirate exams were negative for Fasciola ova. In the absence of adequate serologic diagnostic for fasciolosis in Serbia, the diagnosis was confirmed using enzyme immunoassays and immunoblot at the Institute for Tropical Diseases in Hamburg. Soon after triclabendazole was administered, the symptoms disappeared and biochemical values returned to normal.
CONCLUSION The diagnosis of human fasciolosis may be problematic and delayed, especially in non endemic areas, because physicians rarely encounter this disease and a long list of other diseases must be considered in the differential diagnosis. Unclear history does not rule out fasciolosis.

P.1.12.005 (B)

Multiplex PCR for differential diagnosis of four medically important echinostomes in Southeast Asia

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INTRODUCTION There are four species of echinostomes that commonly infect humans in Southeast Asia, namely *Echinostoma malayanum*, *Echinostoma revolutum*, *Echinostoma ilocanum* and *Hypoderaeum conoideum*. It is difficult if not impossible to differentiate among the eggs or immature stages of echinostome species using morphological characters. The variety of DNA sequences have been used effectively to differentiate among echinostome species. Thus this study aims to develop species specific primer for differential diagnosis of those four medically important echinostomes by multiplex PCR technique.
MATERIALS AND METHODS The specific reverse primers of each species were designed from the variable regions of mitochondrial NADH dehydrogenase subunit 1 gene. All four reverse primers together with a forward primer were used to amplify ND1 fragments. Specificity was tested with others pathogenic helminths, protozoa and bacteria available in the laboratory. Sensitivity of each primer was also evaluated.
RESULT The different PCR product sizes, namely 108, 209, 387 and 417 bp were amplified by species specific primer of *E. revolutum*, *H. conoideum*, *E. ilocanum*, and *E. malayanum*, respectively. No cross amplification between each species was observed. The specificity and sensitivity of each primer was very high at 100% and 10^{-6} to 10^{-8} ng, respectively. When combined all primers together for mPCR reaction, sensitivity of primer to amplify *E. revolutum* was reduced to 10^{-4} ng. The assay system could detect each of a single echinostome egg in 100 mg of normal human feces.
CONCLUSION The species specific primers developed in this study could be consequently used for potentially differential diagnosis of human echinostomiasis in Southeast Asia. Moreover, this multiplex PCR diagnosis could be fruitfully applied for epidemiology study in wild and domestic animals.

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P.1.12.006 (B)

Genetic structure of Southeast Asian Echinostoma revolutum based on mitochondrial CO1 sequence

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INTRODUCTION *Echinostoma revolutum* is a foodborne zoonotic intestinal trematode, causative agents of human echinostomiasis and distributed worldwide. It is a member of the '37-collar-spined' or the 'revolutum' group. This group is a morphospecies and is considered to be a species complex. Previous molecular studies have demonstrated a high level of genetic diversity within a species, *E. revolutum*. Then this study aim to explore the genetic structure of *E. revolutum* in Southeast Asia and also compare to the American and European strain.
MATERIALS AND METHODS Totals of 41 adult *E. revolutum* from Thailand and Lao PDR were collected from domestic ducks. Partial sequence of cytochrome c oxidase subunit 1 (CO1) gene was sequenced. Phylogenetic tree and haplotype network were constructed by Phylip and the Network program, respectively. Genetic structure parameters were calculated based by using Arlequin program.
RESULT The 28 variable positions of CO1 sequence were observed when compare among *E. revolutum* from Southeast Asia. The 11, 14 and one haplotypes of Southeast Asian, American and European strain were subsequently classified, respectively. Three genetic structure groups were demonstrated. Of these, the first group consists of four haplotypes of Southeast Asian strain and closely related with the second group of 14 haplotypes of American strain. The third group consists of seven haplotypes of Southeast Asian strain, which was clustered together with a haplotype of European strain.
CONCLUSION At least three genetic structure groups of *E. revolutum* have been distinguished. Of these, the Southeast Asian strains were classified into two distinct groups. This finding highlight the need for further analyses of intraspecific variation based on molecular and morphological evidence of *E. revolutum* for more understanding of their genetic relationships and genetic structure.

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1.13 HIV/AIDS

P.1.13.001 (B)

Bystander impairment of HIV-specific immune responses by co-incident filarial (*W. bancrofti*) infection

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Many areas of the world struck hardest by the HIV epidemic also bear the heaviest burden of helminth infections (including the tissue invasive filariae), with the result that HIV/helminth co-infections are not uncommon. Several studies have examined clinical outcomes in HIV/helminth co-infected individuals, but none of these studies has focused on the underlying mechanisms of immune interactions. In the current study, we used cryopreserved samples from HIV-positive individuals with (HIV+Wb+; $n = 6$) or without (HIV+Wb−; $n = 9$) *W. bancrofti* infection in South India to interrogate the effect of filarial co-infection on HIV-specific T cell responses. Using multiparameter flow cytometry, we examined cytotoxic potential (perforin and CD107a) and cytokine production (IFN-γ, IL-2, MIP-1α) in CD4+ and CD8+ T cells in response to an HIV-Gag peptide pool, and also evaluated the expression of molecules associated with exhaustion (e.g., PD-1). CD8+ T cells represented a significantly greater proportion of total T cells in HIV+Wb+ individuals compared with HIV+Wb− individuals ($P = 0.0176$), and these T cells appeared to be more activated globally: they had a smaller naïve T cell compartment, higher expression of cytotoxic markers, and more spontaneous cytokine production. CD8+ T cells from HIV+Wb+ individuals had a greater prevalence of PD-1+ cells than did CD8+ T cells from the HIV+Wb− patients (41.4% vs. 21.9%) a feature associated with exhaustion following chronic activation. Furthermore, although mitogen-induced responses were not different between the two groups, Gag-specific CD8+ T cell responses were markedly different: Gag stimulation caused a decrease in the frequency of IFN-γ+ and MIP-1α+ cells among CD8+ T cells from the HIV+Wb+ group (by 17% and 3%, respectively), whereas it induced increases in these cytokine-positive populations by 54% and 135%, respectively, among CD8+ T cells from the HIV+Wb− group. Taken together, these findings suggest that filaria infection induces bystander impairment of HIV-specific immune responses.

P.1.13.002 (B)

Alternative markers to replace clinical staging in the decision to start anti-retroviral therapy (ART) in children when CD4 counts are not available

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Introduction The start of anti-retroviral therapy (ART) is based on CD4 count, however in resource poor settings the costs and complexity of these test limite it's applicability. Therefore, clinical staging is often the only criterion used to start ART. Several alternative markers (haemoglobin, total lymphocyte count (TLC), thrombocyte count, serum albumin and growth parameters) have been suggested to replace CD4 count, but have not been evaluated together in a single study. We evaluated these alternative markers and aimed to identify an algorithm to start ART in HIV endemic areas were CD4 assay is not available.
Methods A prospective cross-sectional study was conducted among HIV-infected Malawian children (18 months–18 years) who were, based on clinical staging, not yet eligible to start ART. Associations between the alternative markers and CD4-counts/-percentages were tested using univariate and multivariate analysis.
Results Of the 417 children who were not eligible to start ART based on clinical staging, 225 (54%) had CD4-count/percentage that required ART commencement. In children under 5 years of age, TLC and height for age *z*-score were associated with CD4 percentage in a multivariate model (beta = 0.2, $P = 0.006$ and beta = −0.2, $P = 0.005$ respectively). In older children (≥ 5 years), only the TLC was associated with CD4 count (beta = 0.5, $P < 0.001$). Sensitivity analysis showed low sensitivity (34%) for TLC among all children and a sensitivity of 53% in the subgroup of children over 5 years of age. Optimizing the WHO TLC cut-offs was useful for older children with sensitivity increasing from 53% to 83%.
Conclusion The use of clinical staging alone to determine commencement of ART will miss a substantial number of children who need treatment. In areas where CD4 counts are not readily available, TLC may be an alternative marker in older children (≥ 5 years) however WHO cut-offs for TLC need to be adjusted.

P.1.13.003 (B)

HIV infection in elderly (above 50 years old) epidemiological, clinical, therapeutical and sociocultural aspects

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Introduction HIV infection is very common in Africa from youth to adult including the infants, by mother to child transmission. The aim of our study is to highlight the epidemiological, clinical, therapeutic and socio-cultural aspects of the disease in elderly, especially for patients above 50 years old which sometimes provoke serious family problems.
Material and Methods Our study is a retrospective one based on the use of patient records monitored on Anti-retroviral therapy (ART) in the period from October 2004 to July 2011 as part of study in our department of medicine.
Results It appears from our study that: 102 cases of AIDS among persons aged more than 50 years were reported from a total of 434 AIDS patients which is 23.50% of the total cases. The age group from 50 to 54 years old is the most affected with 54.9%. 31.7% of our patients had a history of blood transfusion and 26.5% a history of STDs (hepatitis B, gonorrhea). At admission 13.73% of our patients had severe anemia and 74.42% had a CD4 level collapsed <200/ml. However in our study, the unemployed (farmers, housewives) dominate with respectively 22.5% and 23.5%. Over 97% of our patients did not know the HIV status of the spouse before marriage. Our series includes many polygamous and widowers due to a large spread of the disease. Many of our patients are illiterate (57.84%) and had wide families.
Conclusion The strategies for educational and socio-cultural sensibilisation should be done on all levels to avoid late elderly contamination with very dramatic consequences in the family.

P.1.13.004 (B)

The effect of HIV on lymphatic filariasis

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Background Co-infections of *Lymphatic filariasis* (LF) and HIV are seen in several countries, however the influence of HIV on the debilitating manifestations of LF, such as elephantiasis and hydrocele, have not been widely studied.
Objectives Comparison of LF prevalence, filarial worm burden, response to LF treatment and development of chronic pathologies in HIV positive and negative individuals in a cohort in South-Tanzania.
Methods From 2006 to 2011, a population based survey was conducted in the Mbeya Region, Tanzania. In 2009 and 2011, before and after two treatment rounds with ivermectin and albendazole, 1052 sera were tested with TropBio® Og4C3 ELISA, which detects circulating filarial antigen (CFA), HIV status was determined with SD-Bioline strip test and confirmed with ELISA.
Results In 2009, CFA was detected in 41.6% of participants aged 15–94 years; the HIV prevalence was 15.9%. CFA prevalence was similar in HIV negative and HIV positive individuals, 41% vs. 42%, respectively, as was the mean level of CFA, demonstrating similar worm burden in both subgroups. After 2 years of treatment the prevalence dropped significantly ($P < 0.05$) to 32% in HIV negative and 31% in HIV positive individuals. Development of elephantiasis occurred in 16 (3.2%) of HIV negative and 2 (1.6%) of HIV positive participants (RR = 1.83, 95% CI = 0.54 to 6.22). Hydrocele was found exclusively in HIV negative individuals, with a prevalence of 1%. Cytokine measurement in unstimulated PBMC demonstrated a higher level of IL-17 in LF- infected individuals with elephantiasis, compared to LF- infected, but clinically healthy individuals.
Conclusions HIV-infection does not influence LF prevalence, worm burden or treatment success. Chronic pathologies (CP) might develop less often in HIV infected individuals. Together with the more 'aggressive' cytokine –profile in patients with CP, this could suggest a role of the host immune system in the development of LF sequelae.

1.13.1 Global HIV issues in pursuit of universal access and MDG 6

P.1.13.1.001 (B)

Knowledge, attitudes and beliefs in female inmates regarding HIV infection: a survey in a prison in Mali

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Background The prevalence of HIV infection in prisons is higher than in the general population. Few data are available on HIV infection in incarcerated women in Africa. In order to optimize HIV prevention measures in female prisoners, we conducted a study to assess the knowledge, attitudes and practices of the inmates regarding HIV infection.
Methods Descriptive study, conducted from May to July 2012 among females in Bollé's prison (Bamako) incarcerated for more than 3 months. Data were collected during anonymous individual interviews. Univariate analysis was used to determine a significant association between sociodemographics, HIV infection knowledge and risk practices.
Results Eighty-five prisoners with a 22 year-old median age (range: 18–70) were included. Participants had equal to or less than high school level education. Sixty seven (79%) prisoners had at least a child. Only 46% of them knew the virus of the human immunodeficiency. The average score of knowledge was significantly related to the length of incarceration and originally urban of the prisoners ($P < 0.001$). Misconceptions about mode of transmission were identified. The use of the male condom was considered as the essential prevention measure by 63% of participants but was not available in prison. Forty women (47%) had already realized a HIV screening test of which half during the confinement. Stigma towards seropositive persons were observed, only 34% of participants agreed to wear the clothes of a seropositive person. During the confinement, one third of participants have said to practice homosexuality and 72% of them were sharing sharp objects.
Conclusions There was little knowledge about HIV in this prison. Sexual risk behaviors and stigma towards HIV individuals were frequent. Implementation of educational programs on HIV infection and a better access to HIV prevention measures are urgently needed in women's prisons in Mali.

P.1.13.1.002 (B)

Current prevalence, incidence, and residual risk of infectious diseases markers among blood donors in Burkina Faso, 2008–2011

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Background Although routine monitoring of trends in transfusion-transmissible infections (TTIs) is essential to maintaining and improving transfusion safety, there is no trend analysis for TTIs in Burkina Faso. This report presents trend in TTIs obtained among Burkina Faso first-time donors (FD) and repeated donors (RD) collected in 2008 through 2011.
Methods Data collected at four blood centres ($N = 228\,857$) and relating to all blood donations have been analyzed. The prevalence of confirmed-positive test results for hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, and syphilis were evaluated for each year for FD from 2008 through 2011. Incidence rates for these infections were evaluated among RD having at least two donations in a 2-year period. The relationship risk = (window period) × (incidence) was used to assess residual risk among RD.
Results The overall seroprevalence of HIV, HBV, HCV and syphilis was 2.06%, 13.8%, 6.8% and 2.6%, respectively. A significantly increasing trend over time was observed for syphilis and HCV ($P < 0.001$). Syphilis increased from 2.2% (95% CI, 2.1–2.4) to 4.1% (95% CI, 3.9–4.3) and HCV from 6.0% (95% CI, 5.7–6.3) to 7.9% (95% CI, 7.7–8.2). A significantly decreasing trend over time was observed for HBV, from 14.3% (95% CI, 13.9–14.7) to 13.2% (95% CI, 12.9–13.6), and no change for HIV. Incidence rates for all markers of hepatitis declined significantly over time: in 2010–2011, the incidence

rates per 100 000 persons-years were 39.0 for HIV, 44.1 for HCV and 122.5 for HBV. Estimates of residual risk in donations from RD for HIV, HCV and HBV were 1 per 46 729, 1 per 8921 and 1 per 4380, respectively.
Conclusion HCV and syphilis poses a threat to society due to its increasing trend. These data also suggest that integrity of patient identification among FD deserves more attention.

P.1.13.1.003 (B)

Risk behaviors and HIV prevention access among male prisoners in Burkina Faso

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Background In Burkina Faso, the prevalence of HIV infection is five times higher in prisons than in the general population. Few data exist on the vulnerability of prisoners to HIV. The aim of the study was to evaluate knowledge on HIV, attitudes towards the HIV-positive individuals, access to prevention and risk behaviors of male prisoners.
Methods A cross-sectional descriptive study was performed at Ouagadougou prison from June to August 2012. We included male prisoners aged 18 years and older with a more than 3 month stay in prison. Questionnaires were administered to them during interviews in the parlor and two focus groups were conducted with the staff.
Results One hundred and sixty-five inmates were interviewed: 28 year median age, 45% illiterate, 49% married. Median duration of incarceration was 19 months. A small proportion (16%) had a good knowledge on routes of HIV transmission. Mistaken beliefs were frequent (58% thought that mosquito bites transmit HIV). Before incarceration, they had casual sex with many partners and low condom use. While in prison, 4% of inmates reported homosexual relations. The majority (80%) of prisoners was intolerant of HIV infected persons [risk factors: from rural area and illiteracy ($P < 0.0$)]. Around 49% of prisoners shared razors or blades. No one declared injected drug use and tattooing in prison. Only 6% were made sensitive to HIV while incarcerated and 5% were HIV tested in prison. There is a denial of homosexual practices by inmates and prison administration. Finally, condoms were forbidden and not available in this prison.
Conclusion Prison conditions and denial of homosexual practices in Ouagadougou increase the vulnerability of prisoners to HIV. Implementation of a prevention and management program of HIV would significantly reduce the risk of transmission among prisoners, but also discrimination toward them.

P.1.13.1.004 (B)

Challenges in diagnosis, treatment and follow-up of HIV infected patients in Guinea-Bissau

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Introduction Despite support from Global Fund and other donors massive infrastructural problems associated with delivery of anti-retroviral treatment (ART) of HIV in low-resource settings persist. There is a need to solve these problems to improve the outcome for the patients.
Methods From 2005 to 2013, a total of 5449 HIV infected patients have been diagnosed with HIV at Hospital National Simão Mendes (HNSM) in Bissau, the capital of Guinea-Bissau. The HIV clinic is run in collaboration between local doctors, the National HIV Programme, and Danish researchers. We identified infrastructural problems at the clinic related to ART.
Results (i) Inadequate drug supply: Among 3105 patients on ART 485 (16%) have experienced at least one episode of switch of ART due to drug stock outs. Three cases of Stevens-Johnson syndrome were seen within a 5 week period due to switching from Efavirenz to Nevirapine. (ii) Relocation: The clinic has been relocated four times. This can be a contributory factor to the high number of patients lost to follow-up of 60%. (iii) Laboratory incapacity: (a) Diagnostics: Inadequate tests discriminating between HIV-1 and HIV-2 is one of the explanations for 9.3% of HIV-2 infected patients being treated with an insufficient NNRTI based regimen. (b) Biochemistry and CD4 measurements: Major interruptions in availability contribute to delayed initiation of ART, late diagnosis of treatment failure and lack of diagnosis of adverse events. (c) HIV-RNA: No availability of viral load measurement or resistance testing increases the risk of late diagnosis of treatment failure.
Conclusion The largest HIV clinic in Guinea-Bissau faces a number of struggles, which hampers the goal of delivering ART at sufficient quality hazarding the lives of the patients. If similar issues are faced in the many ART facilities in Africa from where few data are reported, the effect of ART roll-out may be impaired.

P.1.13.1.005 (B)

Why do low HIV prevalence contexts in Africa often fail to reach effective ART coverage? Lessons learned on programmatic and delivery model adaptations in Guinea and Democratic Republic of Congo

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Introduction Countries with highest HIV prevalence reach reasonable coverage of antiretroviral treatment (ART), while several low prevalence countries struggle. Low ART coverage implies excess mortality and morbidity, and also limits potential reduction of HIV transmission. Possible hypotheses for this divergence point to ill adapted standard approaches and resistance to innovative approaches proven successful to ART roll out.
Material and Methods We investigated what service delivery factors were perceived as key obstacles to boost ART coverage in low prevalence countries, with a focus on service integration, decentralization, task shifting. This review was done based on existing literature and key interviews of patients, health workers and experienced program officers. Additionally outcomes under differing ART delivery are shown in two urban MSF project sites.
Results and Conclusions Information collection and analysis is ongoing; these are preliminary results. In contrast to high prevalence areas, dedicated ART services might be better in low prevalence settings, as allowing better quality of care through experienced clinicians and adapted support services. Stigma leads to lower uptake in general services, often perceived as less confidential. Moreover, health systems' performance in both DRC and Guinea is weak. Forced integration of HIV

diagnosis & care into overall health services creates problems in access and quality of care. Both apply cost recovery in the general health system, reinforcing reluctance to reduce unnecessary clinic and lab appointments for stable patients. Urban availability of medical doctors creates reticence towards task shifting and necessary simplification of ART care.
CONCLUSION ART coverage is important for reduction of mortality, morbidity and transmission of HIV, also in low prevalence contexts, where there is little priority given to HIV by governments and donors. There is a need to review critically and adapt recommendations towards optimal ART delivery strategies, including operational research on innovative models.

1.13.2 HIV – treatment adherence in resource constrained settings

P.1.13.2.001 (B)

Cognition abilities and daily life functioning of HIV subjects under antiretroviral therapy

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INTRODUCTION To assess the impact of front line HIV antiretroviral drugs on cognition and daily functioning of HIV subjects.
SUBJECTS AND METHODS Neuropsychological evaluation of 200 HIV subjects (age 40.6 ± 10.6 years, mean nadir cd4 185.9 ± 179.9/ml, and duration of infection ranging 2–18 years). The evaluation was carried out with the HIV dementia (HDS) and the instrumental activities of daily living (IADLs) scales. Subjects under highly neuroactive drugs were compared to those treated with poorly neuroactive drugs as per their central nervous system penetration-effectiveness scores. Statistical analysis was performed using a logistic regression analysis at the significance level of 0.05.
RESULTS The level of education, occupation, nadir cd4, and the type of treatment regimen were significantly associated with the degree of cognitive impairments ($P < 0.01$). Subjects under poorly neuroactive drugs and cognition deficits were more likely to be dependent in their daily functioning, mostly with regards to adherence to treatment regimen ($P < 0.01$). The odds ratios of being under poorly neuroactive drugs or having impaired cognition were 3.4 (95% CI: 1.2–43.3) and 11.8 (95% CI: 2.47–28.08) with respect to functional dependency; or 2.8 (95% CI: 1.11–7.15) ($P < 0.01$) for poorly neuroactive drugs after adjusting for treatment regimen.
CONCLUSION Poorly neuroactive HIV antiretroviral drugs are associated with poor cognition, dependency in daily functioning of subjects, and risk for non-adherence to treatment. These findings need to be integrated with global health policies to control the HIV pandemic.

P.1.13.2.002 (B)

Psychometric properties of Mooré version of WHOQOL-HIV BREF in patients with HIV infection in Burkina Faso

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With prevalence at 1.1%, HIV remains public health problem in Burkina Faso. The aim of the current study was to examine psychometric properties of mooré version of World Health Organization's Quality of Life assessment tool in HIV patients (WHOQOL HIV-BREF). Cross-sectional study was conducted on 100 persons living with HIV/AIDS in a community based organization's health facility. Named Association des Jeunes pour la Promotion des Orphélins (AJPO), it's located in Ouagadougou, capital city of Burkina Faso. The study showed high internal consistency with Cronbach's alpha at 0.92 for the whole tool. According to domains, Cronbach's alpha found was ranged from 0.58 to 0.87, showing acceptable or good internal consistency for all domains. Domains scores for test-retest reliability, using Interclass Correlation gave coefficients ranged from 0.66 (Psychological domain) to 0.99 (Level of Independence domain) with $P < 0.001$ for all domains except for spiritual domain (ICC = 0.40; $P < 0.05$). Spearman's correlation showed significant correlation between general facets and overall quality of life score ($P < 0.001$). General facets and six domains of quality of life showed also significant correlation ($P < 0.001$), except for spiritual domain. The tool demonstrated good discrimination according to clinical stages of HIV infection with higher scores in HIV asymptomatic patients and worst scores in AIDS stage patients ($P < 0.001$), except for spirituality domain ($P > 0.05$). As a demonstrated cross-cultural tool, the WHOQOL-HIV BREF in its Mooré version can be used for quality of life assessment in a routine way or longitudinal studies in Burkina Faso with persons living with HIV/AIDS.

1.15 Drug quality

P.1.15.001 (A)

Association of azole resistance in *C. glabrata* with overexpression of fatty acid activator 1 gene

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INTRODUCTION *Candida glabrata* (*C. glabrata*) causes serious systemic infections in immunocompromised individuals, and many strains of this organism are naturally resistant to azole antifungal drugs. The aim of this study was to investigate the molecular mechanisms of azole resistance in clinical isolates of *C. glabrata*.
MATERIAL AND METHODS The clinical strains were selected from an epidemiological survey of candidemia in immunosupprressed patients, including of two fluconazole and itraconazole susceptible isolates and four fluconazole and

itraconazole resistant isolates and *C. glabrata* CBS 138 as reference strain. Antifungal susceptibility patterns of clinical isolates were tested by Clinical and Laboratory Standards Institute (CLSI). Complementary DNA Amplified Fragment Length Polymorphism (cDNA-AFLP) technology and semi quantitative RT-PCR were developed for identified the potential gene(s) which involved in the molecular mechanisms.
RESULTS The results obtained that fatty acid activator 1 (FAA1) gene, belonging to Acyl-CoA Syntheses, was upregulated in the resistant clinical isolates using cDNA-AFLP method. Semi-quantitative RT-PCR determined that FAA1 gene expression increased in resistant clinical isolates (1. ≥ 2 fold) as compared to susceptible isolates and *C. glabrata* CBS 138 reference strain.
CONCLUSIONS This study revealed that high expression of the FAA1 gene in lipid metabolism provided the azole resistance in clinical *C. glabrata* isolates.

Track 2: Reproductive and child health

2.1 Reproductive health and sexual rights

P.2.1.001 (B)

The role of ubiquitin-proteasome system in trichomonas vaginalis under iron stress

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Trichomonas vaginalis is the causing agent of trichomoniasis, the most prevalent sexually transmitted infection in humans. Previous studies showed that many biological and pathological processes in *T. vaginalis* are iron dependent. However, the mechanism of iron-regulated gene expression network is still largely unknown. In the present study, we investigated the effects of iron on the growth of *T. vaginalis*. The parasite exhibits growth arrest and transforms to pseudocyst in the presence of the permeable iron chelator dipyridyl (DIP), speculating iron is crucial for proliferation of this protist. To analyze the mechanisms that required for survival under iron stress, we employed next generation sequencing technology to compare the transcriptome of *T. vaginalis* grown in different iron availabilities. We found that ubiquitin, a major component of the proteasome-dependent proteolysis system is highly expressed in iron-deficient cells. In order to elucidate the roles of ubiquitin-proteasome system (UPS) in iron-stressed *T. vaginalis*, we monitored the growth of cells treated with proteasome inhibitor, MG132. The growth curve showed that proteasome is important for life maintenance in iron-deficient group compared with MG-132-untreated group. Iron-deficiency might cause cellular reactive oxygen species (ROS) accumulation because ROS scavengers require iron as electron acceptor. We showed that the intracellular ROS level in iron-deficient group is higher than iron-rich group, but the highest level is shown in MG-132 treated *T. vaginalis*. Thus, one of the roles of UPS in *T. vaginalis* is ROS clearance. These data indicate that UPS is crucial for *T. vaginalis* survival under iron stress, which might provide a new insight for new drug development.

P.2.1.002 (B)

The effects of community-based reproductive health workers on the utilization of family planning services in Yemen

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This study focuses on community health workers who were trained within the project of community-based reproductive health promotion (CBRHP). The purpose of this study is to explore the effects of community-based intervention on utilization of family-planning services in communities which implemented CBRHP.
METHODOLOGY A cross-sectional comparative study was conducted in two villages with intervention of the CBRHP program and two non-intervened villages. The study was conducted after 19 months from the intervention date. Stratified proportional random sample was applied for the selection of the household. Equal numbers of participants were interviewed with questionnaire from both study arms with involvement of both sexes. Data analysis was made interpreted with odd ratios (ORs) and 95% confidence interval (CI) additionally, multivariate analysis was included too.
FINDINGS The rate of ever use of modern contraceptive methods is higher among people in the intervention areas (74.5%) in comparison to the non-intervention areas (51.1%). Oral contraceptive pills were the most commonly used contraceptives among women in both areas. However, in the intervention areas the usage was higher than the non-intervention areas (63.7% and 27.7% respectively). Women in rural areas prefer the use of a 3 months injection rather than visit a health facility monthly to get one strip of oral pill. Families' with children younger than 2 years are using contraception more than those who have children older than 2 years (OR = 4.37, (1.79–10.64) with 95% CI $P = 0.001$). The age of the last child adjusted ratio (AOR = 2.62) is less than the crude OR (3.41) indicate that the age of the last child differences between the exposed and non-exposed areas are taken into account.
CONCLUSION The study concluded that CBRHP volunteers contributed to provide contraceptive methods for more than two thirds of people in the intervention rural areas.

P.2.1.003 (B)

What promotes sexual and reproductive health knowledge among youth in India?

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The increasing incidences of reproductive tract infection and sexually transmitted diseases including HIV/AIDS are a great challenge today especially for developing countries. Due to consequences of sexually transmitted infections and diseases, it is necessary to increase awareness on reproductive and sexual health matters among unmarried youth. Thus, an attempt here is made to identify the sources through which youth get information on reproductive and sexual health issues and which sources need to be focused more in promoting the reproductive and sexual health awareness among youth. Data for the analysis come from a cross-sectional survey 'Youth in India: Situation and Needs 2006–2007' conducted by International Institute for Population Sciences (IIPS), Mumbai and Population Council, New Delhi, India. Analysis has been done here using bi-variate and

multivariate techniques. Multinomial regression has also been applied to examine the major sources of information on sexual and reproductive health matters among youth. The study reveals that majority of youth get information on reproductive and sexual health through their friends and peer networks. Mass media is the second most commonly used source of information on several issues related to reproductive health. It is important to mention that parentâ€™s of youth plays very negligible role in increasing awareness among youth in India. Very few parents have developed communication regarding sexual and reproductive health with their young children. It may be because of existing cultural barriers and lack of education and knowledge limit them doing so. To some extent, school/teachers and health care providers are also supportive in promoting awareness among youth on the issue. The study reveals that there is a need to have proper policy and educational programs for youth as well as for their parents so that they can able to spread the awareness among youth regarding sexual and reproductive health matters.

P.2.1.004 (B)

Reproductive health and rights of teens in South Africa – prevalence of teen pregnancy, forced sex and contraceptive use amongst KwaZulu-Natal high school students

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INTRODUCTION Despite policies providing free contraception and termination of pregnancy, teen pregnancy and sexual abuse are a serious concern in South Africa. The study aimed to investigate the prevalence of teen pregnancy, forced sex and use of contraception amongst students attending schools in KwaZulu-Natal.
METHODS A cross sectional descriptive study was undertaken in 20 randomly selected urban (eThekwini) and rural (Ugu) district schools. Male and female grade 11 students ($n = 662$) completed an anonymous questionnaire about teenage pregnancy and contraceptive practices.
RESULTS Of 294 male (44.4%) mean age 17.85 years (95% CI 16.68, 18.02) and 368 female (55.6%) students, mean age 17.30 years (95% CI 17.15, 17.45), 269 (40.6%) were sexually active and of these 70 (10.6%) had either caused a pregnancy, 27 males, (9.2%) or been pregnant, 43 females, (11.7%). Ever pregnant/caused a pregnancy was associated with current contraceptive use ($P < 0.005$). Use of male condoms was reported by 166 (61.7%) of sexually active students (74.5% of males and 41.3% of females). Students (23, 8.6%) reported use of the birth control injection, female condom, (15, 5.6%), and birth control pills (12, 4.5%). Dual protection, injection plus male condom (10, 3.7%) or contraceptive pill plus male condom, (7, 2.6%) was reported. Rural females were significantly less likely to use male condoms ($P < 0.005$). No association was found with forced sex (4.8% of total sample), reported by 15 males (5.1% of males) and 17 females (4.6% of females). Condom use at last sex was reported by 143 (53.2%) and consistent condom use by 123 (45.7%) students.
CONCLUSION Despite prevention efforts to reduce teen pregnancy and the high prevalence of HIV/AIDS, many sexually active students do not use condoms. New approaches are needed to address teen contraception and sexual abuse in order to improve the reproductive health of high school students.

P.2.1.005 (B)

Factors influencing the sexual and reproductive health of the teenagers in rural areas in Haiti

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INTRODUCTION The sexual and reproductive health (SRH) of the teenagers is a key element of the Millennium Development Goals objectives (MDG5 and MDG6). Indicators of SRH remain alarming despite the adoption of a national health policy for youths and teens by the Haitian government in 2001. Thus, the objective of this study was to assess factors influencing sexual risk behaviors among rural adolescents between 15 and 19 years old.
METHODS This was a cross-sectional and descriptive study by means of a questionnaire on a sample of resident teenagers in a rural zone of Haïti (Fond-des-Blancs) drawn lots in six localities.
RESULTS One hundred and fifty teenagers (88 females, 62 males) middle-aged 16 years were interviewed. The level of knowledge towards the STIs and HIV was low. Two thirds of the teenagers had already had a first sexual intercourse. The average age in the first sexual intercourse was 11 years (range: 7–15 years). The condom was the most common form of contraception used (42%), while more than half (51%) used no form of contraception method. Among the females, three (2%) had at least one child while three (2%) said they had an abortion. Information sources on the sexuality were the radio, the community groups and the television. Despite our findings, there were no longer any educational programs nor reproductive health services for adolescents in the area. Free methods of contraception were not available.
CONCLUSION This pilot study confirms the sexual risk behaviors of the Haitian teenagers living in rural area. Poverty, lack of information and inaccessibility of the SRH explain this situation. The sexual education of rural adolescents in Haiti should be strengthened as well as access to services for SRH. These services would play a role in preventing STIs and unwanted pregnancies among adolescents in rural Haïti.

P.2.1.006 (B)

Does intimate partner violence influence the utilization of maternal health care among women in India?

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INTRODUCTION Intimate partner violence (IPV), a common form of violence against women perpetrated by husband or intimate male partner, often happens in low developed countries. IPV is found to be associated with range of negative effects: often leads to still birth, premature delivery, low birth weight, high risk of RTI/STI etc. In India, at least half of battered wives reported the episode of violence while they were pregnant. However, there is no clear understanding about the association between IPV and utilization of maternal health care services in the context of India.
OBJECTIVE The present study examines the association between IPV and utilization of prenatal care and skilled birth attendance among women in India and its states that starkly vary in socioeconomic and demographic parameters.
DATA AND METHODS The study uses data from third round of National Family and Health Survey conducted during 2005–2006. The original sample size is restricted to currently-married women of reproductive age who completed the surveys Domestic

Violence Module and who had a pregnancy in the past 5 years. Using adjusted predicted probabilities from the multiple logistic regression models, the study compares the utilization of prenatal care and skilled birth attendance by exposure to various types of IPV.
RESULTS AND CONCLUSION Women who ever experienced IPV showed consistently attenuated probabilities to seek maternal health care services in India. After adjusting for socio-demographic covariates, the study found that women with a history of intimate partner violence reported significantly lower probabilities to opt for prenatal care. However, skilled birth attendance was not found to be significantly associated with IPV. The study stress the need for developing policies and programs that integrate reproductive health and IPV components to improve the uptake of essential maternal health care among women living with abusive partners in India.

P.2.1.007 (B)
Prevalence of *Trichomonas vaginalis* among pregnant women in Kinshasa, Democratic Republic of the Congo

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Trichomonas vaginalis (Tv) is a flagellated protozoan causing sexually transmitted infection extremely common among the poor. It is cosmopolitan and is spread when hygiene measures are defective. It is also a cofactor in the transmission of HIV, a major cause of vaginitis in pregnant women and responsible for premature delivery, premature rupture of membranes, low birth weight children and infection of newborn. So we wanted to determine the prevalence of Tv among pregnant women. We conducted a cross-sectional study from November to December 2012 among pregnant women in Kinshasa. A sample of vaginal secretions swab was done, fixed on a slide and stained with Giemsa. Of 412 women enrolled, 96 patients were infected with Tv or 23.3%, whose average age was 28 ± 6 years. The infection was common among married women (55.6%), with a status of monogamous marriage (59.5%) whose spouse was trader (34.5%).

2.2 Child health

P.2.2.001 (A)
Skin disorders in school children in a rural area in Southern Ethiopia

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INTRODUCTION Skin disorders are very common in children in low-income countries. We decided to study the prevalence of skin disorders in school children in a primary school in a rural area in southern Ethiopia.
MATERIAL AND METHODS We involved in the project an Ethiopian primary school. Our team consisted of two dermatologists and two interpreters. All children were interviewed and examined naked in good day light.
RESULTS A total 647 students were interviewed and examined, 329 (50.9%) of whom were boys. The age range was from 4 to 15 years, with a mean age of 10.0 2.4. Of the students examined, 87 (13.4%) had layers of dirt all over the body and 498 (77%) had the lower part of their legs and feet covered in dry mud. Sixty-four (9.4%) students were barefooted. Only 8 (1.2%) students had trivial marks. Of the 647 children examined, 585 (90.4%) presented skin disorders. One hundred sixty five had one dermatological problem, 207 had two skin disorders and 213 had three or more skin problem. A total of 194 (30%) patients had postinflammatory hyperpigmentation, 184 (28.4%) students had onychodystrophy of foot nails, 159 (24.6%) had tinea capitis, 56 (8.7%) had tinea pedis and 50 (7.7%) had tinea corporis-faciei. Scars were observed in Hundred fifty two students (23.5%), mainly secondary to injury (n = 118; 77.6%) and fire burn (n = 44; 28.9%). Hundred twenty four students (19.2%) had pediculosis capitis, 43 (6.6%) had warts, 41 (6.3%) had calluses, 35 (5.4%) had café-au-lait macules, 32 (4.9%) had scabies, 27 (4.2%) had xerosis and 25 (3.9%) had nevus.
CONCLUSION Fungal infections, postinflammatory hyperpigmentation and onychodystrophy of foot nails were the most common skin diseases in our study population, whereas inflammatory illnesses were nearly absent.

P.2.2.002 (A)
Measles elimination: tailoring vaccination approaches in the DRC for better results

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INTRODUCTION DR Congo faces an ongoing measles outbreak since end of 2010, whilst more than 80% of the health zones (HZ) have been covered by supplementary vaccination campaigns in 2011 and 2012. High vaccination coverage was reported for these campaigns. Médecins Sans Frontières (MSF) has partnered with the Ministry of Health to respond with success to the measles outbreaks in several HZs. Mainly through tailoring implementation to specific local context factors, a sharp reduction of measles cases and high vaccination coverage – as verified by population based surveys – were obtained.
MATERIAL AND METHODS A comparative review was made of the HZ data for 2012, including: inputs in terms of resources, implementation effectiveness of vaccination, number of measles cases treated (as reported by health structures) and the coverage results obtained by population based surveys (pre- and post-intervention).
RESULTS AND CONCLUSION Reported vaccination coverage following the 2011 campaign did not reflect the coverage as measured at population level: <40% of children reported immunisation. Support by MSF in 2012 targeted additional resources to increase effectiveness of the intervention and adaptation of the standard national plan to local problems. Waiving patient fees allowed for increased health service utilisation and low lethality. High immunisation coverage was obtained thanks to targeted reinforcement with additional staff and logistic material to organise quality outreach activities, reaching the population not using the existing health services. Experience in DRC shows that effective immunisation services need to take into account local context factors and to adapt implementation strategies accordingly. To reach the most vulnerable in particular, fixed sites based campaigns do not meet their objective, especially when existing health services are underutilised. Additional resources targeted towards outreach and better financial access are indispensable to include hard to reach populations and ensure effective protection in HZ.

P.2.2.003 (A)

Low birth weight followed by rapid weight gain is associated with increased fatness in Ethiopian infants

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INTRODUCTION Fetal and postnatal weight gain is associated with early survival and later risk of type 2 diabetes (T2D) and cardiovascular diseases (CVD) in low- and high-income populations. However, the underlying early life changes in body composition have not been described. We examined the role of fetal and postnatal weight gain as predictors for body composition at 6 months of age in a low income population.
METHODS In a prospective cohort study body composition was repeatedly measured between birth and 6 months of age using air displacement plethysmography. Birth weight was used to indicate fetal weight gain and weight at 6 months of age adjusted for birth weight to indicate infant weight gain. Fat mass (FM) and fat-free mass (FFM) was divided by length squared to obtain fat mass index (FMI) and fat-free mass index (FFMI).
RESULTS A total of 255 infants were followed from birth to 6 months of age. Infants in the lowest birth weight tertile had less FM and less FFM at birth. If they grew slowly, they maintained lower FFMI at 6 months compared to the reference group (FFMI deficit: −0.70 kg/m^2, 95% CI: −1.21; −0.18, $P \leq 0.01$) (Figure 1). If they grew fast, they resolved the lower FFMI by 6 months, but developed greater fatness compared to the reference group (FMI surplus: 1.18 kg/m^2, 95% CI: 0.64; 1.72, $P < 0.01$) (Figure 2). There was a dose-response association, independent of birth weight, between post-natal weight gain and fatness at 6 months.
CONCLUSIONS Low birth weight babies have less FFM at birth. This deficit can be resolved by a rapid post-natal weight gain, but goes along with an increased fat accretion. Extra catch-up of fat early in life may play a role in the later risk of T2D and CVD among low birth weight infants.

Figure 1: Mean (95%CI, SD) FFMI at 6 months of age by tertiles of birth weight and 0-6 month weight gain

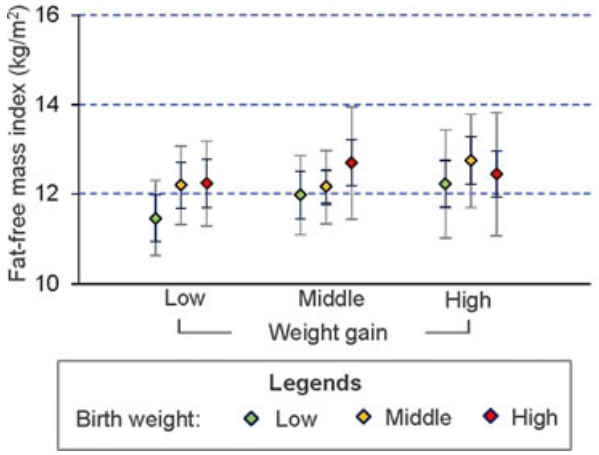
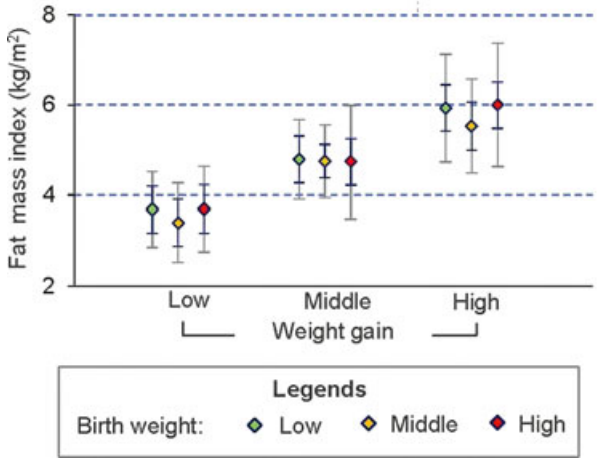


Figure 2: Mean (95%CI, SD) FMI at 6 months of age by tertiles of birth weight and 0-6 month weight gain



2.3 Maternal health

P.2.3.001 (A)

Differences between public and private hospital maternal health service utilization and capacity in Southern Tanzania: using a geographic information system to link hospital to national census data

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INTRODUCTION Tanzania faces high maternal mortality (454/100 000 births) and low rates of skilled birth attendances (50.5%). With 40% of services provided by the private sector, increased collaboration between public and private actors is considered key in reducing unmet maternal health needs. To provide a basis for informed decision making, this study explored differences between public and private hospital maternal health service utilization and capacity in southern Tanzania and discusses resulting policy implications.
METHODS AND RESULTS We assessed all 16 hospitals (public $n = 10$; private faith-based (FBO) $n = 6$) in 12 districts of southern Tanzania. Information included availability of maternal health related infrastructure, human resources and user-fees. We linked quantitative and spatial patient turnover information from paper based patient records to the 2002 Population and Housing Census dataset and a geographic information system (GIS) to visualize patient flows and FBO service contribution by ward. Services assessed included antenatal care ($n = 12\,274$), normal deliveries ($n = 16\,564$) and c-sections ($n = 2658$) (period reviewed: January-December 2008). Poisson regression revealed higher relative service capacity in FBO compared to public hospitals using selected proxy indicators (c-section rate: RR 1.92; 95% CI 1.77–2.08; $P < 0.001$; maternity nurses/1000 births: RR 1.58; 95%CI 1.09–2.28; $P = 0.015$; medical doctors and assistant medical officers/1000 births: RR 1.58; 95% CI 1.05–2.38; $P = 0.027$; maternity beds/1000 births: RR 1.42; 95% CI 1.18–1.70; $P < 0.001$). FBO hospitals also had a higher

relative share of rural patients (ANC: OR 4.40; 95% CI 4.02–4.81; normal deliveries: OR 1.68; 95% CI 1.54–1.83; c-sections: OR 2.83; 95% CI 2.24–3.59).
Conclusions The findings indicate higher relative service capacity in FBO hospitals. The high proportion of rural patients within FBOs stresses the need to extend public user-fee exemptions to the private sector to reduce inequities in access to care. Using GIS to link hospital to national census data is applicable in Tanzania and identifies target populations for future maternal health research and interventions.

P.2.3.002 (A)

Effective coverage of skilled attendance in Brong Ahafo region, Ghana

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Introduction Globally, over 270 000 maternal and 3 million neonatal deaths occur annually; many during the intrapartum period. Delivery in health facilities with skilled attendants is a strategy proposed to reduce this burden. In order for skilled attendance to be a meaningful indicator of progress towards MDG5, quality of care needs to be considered. We utilized a multi-dimensional evaluation of maternal and newborn health service quality to estimate 'effective coverage' of skilled attendance.
Methods The study site covers seven contiguous districts under demographic surveillance in the Brong Ahafo region. We assessed quality of care in all health facilities in the area, considering four dimensions: routine labour and postnatal care, EmOC, emergency newborn care (EmNC) and acceptability of care. We estimated effective coverage of skilled attendance, defined as delivery in a facility with high quality in all four dimensions.
Results Of 15 884 live births in the study area between November 2008 and December 2009, 10 782 (67.9%) were delivered in a facility. Estimates of skilled attendance were far lower when quality of care was taken into account. Considering each dimension individually, between 52% (routine care) and 20% (EmNC) of deliveries were in facilities with high or highest quality care. Only 18% were in facilities with high or highest quality in all dimensions of care simultaneously, and received skilled attendance. Furthermore, effective coverage varied with wealth quintile; 4% of live-births in the lowest wealth quintile were in high quality facilities compared to 37% of live-births in the highest quintile.
Conclusions Estimates of 'skilled attendance' from surveys, as the proportion of deliveries in a facility or with a skilled provider, are too optimistic if quality is not considered. Wider use of similar assessments could help to move from monitoring coverage alone to monitoring 'effective coverage' which may align better with health outcomes.

P.2.3.003 (A)

Health workers' counselling practices on and women's awareness of pregnancy danger signs in selected rural health facilities in Burkina Faso, Ghana and Tanzania

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Background This study assessed health workers' counselling practices on danger signs during antenatal consultation and pregnant women's awareness of these signs and identified factors affecting counselling practices and women's awareness. The study is part of QUALMAT, an intervention research project funded by the European Commission aiming to improve maternal and newborn health. QUALMAT is conducted in Burkina Faso, Ghana and Tanzania.
Methods A cross-sectional study was performed in 12 selected primary healthcare facilities in each country. WHO guidelines were used as standard for good counselling. We assessed providers' counselling practice on seven danger signs through direct observation study (35 observations/facility). Exit interviews (63 interviews/facility) were used to assess women's awareness of the same seven signs. We used negative binomial regression to assess associations with health services' and socio-demographic characteristics.
Results About one in three women were not informed on any danger sign. For most single signs, less than half of the women were counselled. Vaginal bleeding and severe abdominal pain were the signs most counselled on (between 52 and 66%). At study facilities in Burkina Faso 58% of women were not able mentioning a danger sign, in Ghana this was 22% and in Tanzania 30%. Fever, vaginal bleeding and severe abdominal pain were signs most frequently mentioned. Kind of health worker (depending on training) was significantly associated with counselling practices. Depending on the study site, characteristics significantly associated with awareness of signs were women's age, gestational age, gravidity and women's educational level.
Conclusion Counselling practice is poor and not very efficient. A new approach of informing pregnant women on danger signs is needed. Adopting a more client-centred approach might be an option. However as effects of ANC education remain largely unknown it is very well possible that improved counselling will not have effect on maternal and newborn mortality and morbidity.

P.2.3.004 (A)

Mixed-methods evaluation of a maternity care intervention in rural Nepal: measuring what works

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Brief Introduction The Buddhist charity Green Tara Nepal (GTN) set up a programme to improve the uptake of

maternal care practices in rural Nepal via health promotion activities in the community. The programme is novel because it seeks to improve maternal service uptake via bottom-up participatory methods in rural communities. This research aimed to compare the effectiveness of the GTN health promotion strategy, with standard care for mothers in a developing country community setting.
Material and Methods The research was a mixed-methods evaluation of a maternity care intervention in rural Nepal; conducted in collaboration with GTN. Data were collected using a controlled before-and-after, cross-sectional survey; with socio-economic, cost and health uptake questions. A questionnaire-based interview was conducted with 1236 women, with their last child <2 years old. Ethical approval provided by the Nepal Health Research Council. The relation between the number of ANC (antenatal care) visits, belonging to the intervention group, and the respondents' background (education, household income & parity) was examined.
Results The health promotion intervention appeared to improve ANC attendance. Low educational level, low household income, and multiparty were risk factors for non-attendance. The evaluation suggests that practice should be socio-culturally appropriate and inclusive not only of women but also their families; mother-in-laws' and men's participation should be sought.
Conclusions Nepal will be able to achieve most of its MDG targets by 2015, particularly MDG5 and evaluations, like these have implications for future policy; findings can be used to shape policy to be 'inclusive' to those marginalised, for example rural communities in Nepal. Furthermore, as impact evaluations influence policy, here the implications for Nepalese policy is that maternal health promotion is central to achieving maternal health goals nationally and should be part of the nurse midwifery curriculum. Finally, funding was provided from Bournemouth and Santander Universities.

P.2.3.005 (A)

Estimates of maternal mortality in south west-Ethiopia using four different methods

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Introduction Because of lack of vital registration, measuring maternal mortality is difficult in developing countries.
Objective To compare four methods of measuring maternal mortality in rural south-west Ethiopia.
Materials and Methods We used four methods to estimate maternal mortality. (i) From January 2010 to December 2012, using health extension workers, we prospectively registered 39 698 births and birth-outcomes in four provinces with population of about 500 000 people. (ii) In February 2010, we conducted a survey in 6572 households, in 15 randomly selected villages in one district, that had pregnancy and outcomes within 5 years before the survey. (iii) We estimated the lifetime risk (LTR) of maternal mortality and maternal mortality ration (MMR) by interviewing 8503 adults using the indirect sisterhood method. (iv) We reviewed one year institutional records on births and outcomes from all 63 health centres and three hospitals in Gamo Gofa (population 1.8 million).
Results We compare data from 2010. Using birth registration, we recorded 10 613 births and 53 maternal deaths in 2010 (MMR of 499 per 100 000 LB). In the household survey, we counted 11 762 births (11 536 live and 226 stillbirths), and the MMR of 425 per 100 000 LB. The indirect sisterhood method resulted in an LTR of 0.102 with corresponding MMR of 1667 per 100 000 LB. Because of indirect nature of the sisterhood method, the estimate refers to year 1998. The facility review includes 4231 deliveries, 6.6% of 64 413 expected births, and we recorded 79 maternal deaths (MMR 123 per 100 000 LB of expected deliveries in the same year).
Conclusion The community-based estimates (birth registry and household survey) are comparable. The birth registry provides variations of MMR between districts and trends. The indirect sisterhood measure is high. Only 26% of the expected maternal deaths were recorded at the institutions, taking the birth-registry finding as a reference.

P.2.3.006 (A)

Factors affecting antenatal care attendance: results from a qualitative study in Madang, Papua New Guinea

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Background Appropriate antenatal care (ANC) is key for the health of mother and child. However, in Papua New Guinea (PNG), only a third of women attend ANC. Drawing on a programme of qualitative research, this article explores the factors that influence ANC attendance in Madang, PNG.
Methods Data were collected at three sites utilizing several qualitative methods: free-listing and sorting, focus group discussions, in-depth interviews, observation in health care facilities and case studies of pregnant women. Respondents included pregnant women, their relatives, health providers, opinion leaders and community members.
Results Although generally reported to be important (particularly to ensure a safe delivery), respondents' ideas about ANC were vague. There was also a tendency to view ANC as curative rather than preventive. The instances of women who moved house to be closer to a health facility illustrate the importance of accessibility (distance and cost) for ANC attendance. However, other women, who lived close to health facilities and could easily afford ANC, demonstrated poor attendance. Additional factors were therefore also relevant, such as previous experiences of ANC – waiting times, health care workers' attitudes, perceived quality of care – along with pregnancy disclosure, family support and relationships. A desire to avoid repeat clinic visits, ideas about the strength of the fetus and parity were particularly relevant to the timing of women's first ANC visit.
Conclusions Previous studies have focused on accessibility as major determinants of ANC attendance in PNG. However, the findings suggest that additional supply and demand-related issues are also relevant. Interventions to improve ANC attendance could therefore focus on its delivery in health facilities, for example, by targeting the attitudes of health care staff to pregnant women.

P.2.3.007 (A)

Measuring maternal mortality in a census: results of the Zambia 2010 Census of Population and Housing

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Introduction Maternal mortality is a rare event and hence measuring it is a global challenge. In the quest for reliable

Table 1 *In vitro* activity, toxicity and selectivity index found for the derivatives on extracellular and intracellular forms of *T. cruzi*

	IC_{50} (μM)*			IS_{50}†			
Compuestos	Epimastigote forms	Intracellular amastigote forms	Trypomastigotes	Toxicity IC_{50} Vero cell (μM)‡	Epimastigote forms	Intracellular amastigote forms	Trypomastigotes
BZN	15.9 ± 1.1	23.3 ± 4.6	22.4 ± 1.9	13.6 ± 0.9	0.8	0.6	0.6
J20	39.21	20.8 (8)	29.5 ± 6.1	148.76	3.8 (5)	7.1(12)	5.0 (8)
J21	20.60	17.4 (13)	24.5 ± 4.9	205.72	10.0 (12)	11.8 (20)	8.4 (14)
J22	12.78	10.9 (22)	108.7 ± 7.7	214.68	24.6 (31)	28.8 (48)	2.9 (5)
J23	22.86	22.5 (15)	111.5 ± 10.0	298.65	13.0 (16)	13.3 (22)	2.7 (4)
J24	5.60	4.0 (32)	61.1 ± 3.5	112.95	66.6 (83)	93.2 (155)	6.1 (10)

Results are averages of four separate determinations.

*IC_{50} = the concentration required to give 50% inhibition, calculated by linear regression analysis from the Kc values at concentrations employed (1, 10, 25, 50 and 100 lM).

†Towards Cell Vero after 72 h of culture.

‡Selectivity index = IC_{50} Cell Vero/IC_{50} extracellular and intracellular form of parasite. In brackets: number of times that compound exceeds the reference drug SI (on extracellular and intracellular forms of Trypanosoma cruzi).

estimates of maternal mortality to monitor progress towards millennium development goal (MDG) number 5, the United Nations recommended the inclusion of key questions on maternal mortality during the 2010 round of national population censuses.

Methods The Zambia census was conducted between October and November 2010. Household deaths data was collected from all households interviewed. For deaths of females aged 12–49, three questions related to pregnancy and child birth were asked; did the death occur while the woman was pregnant, during childbirth, or during the 6 weeks period following the end of the pregnancy. Demographic methods were used to analyse the data and compute maternal mortality estimates.

Results The observed MMRatio based on all reported pregnancy-related deaths was 691.5 per 100 000 live births. There was a marginal difference in the observed MMRatio for rural (739.9 per 100 000 live births) and urban (613.1 per 100 000 live births). Sensitivity analysis showed a wide range in MMRatios from 345.7 to 1037.2 per 100 000 live births when a 50% adjustment in reported maternal deaths was applied in either direction. The estimates obtained with a 30% downward adjustment in reported maternal deaths, 484 deaths per hundred thousand live births (518 in rural and 429 in urban) were more plausible.

Conclusion The Zambia 2010 census yielded high estimates of maternal mortality. A previous maternal mortality study from Zambia indicated that a 30% downward adjustment of pregnancy-related deaths was needed to obtain a better estimate of actual maternal deaths. We found that such an adjustment yielded estimates that were in line with other robust estimates for the same period. Future censuses must pay greater attention to data quality control measures including use of detailed cause of death codes.

P.2.3.008 (A)

Evaluation of a participatory antenatal care strengthening intervention on health promoting behaviours in Jimma, Ethiopia, 2009–2011

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Introduction Antenatal care (ANC) provides a unique opportunity to counsel women on healthy behaviours. However, often health education is not provided or the time allocated not sufficient. The objective of this paper was to assess the effect of a participatory ANC strengthening intervention on health promoting behaviours.

Material and Methods The intervention was designed participatorily and consisted of trainings, supervisions, equipment, development of health education material, and adaption of guidelines. It was implemented at three health centres and one hospital in the Jimma area. Control sites were included. A questionnaire survey before (2008–2009) and after (2010–2011) intervention was conduced with women, who gave birth within 12 months preceding the interview dates. The effect of the intervention was assessed by comparing the change in health promoting behaviours from before to after the intervention period at intervention sites relative to control sites using logistic regression.

Results There was a positive effect of the intervention on breastfeeding practices (OR 2.0, 95% CI 1.2; 3.2), and preventive health care for the infant (OR 1.9, 95% CI 1.3; 2.8). The proportion of women attending for four visits decreased at intervention sites, but increased at control sites. There was no effect of the intervention on gestational age at first visit and infant immunization coverage. The effect of the intervention on various outcomes was significantly modified by maternal education, and the intervention increased health facility delivery (OR 2.7, 95% CI 1.0; 7.1) and breastfeeding practices (OR 12.0, 95% CI 3.6; 39.6) among women with no education.

Conclusion The participatory intervention translated into improved health promoting behaviours. The improvements seen among the most vulnerable ANC attendants in health facility delivery and breastfeeding is encouraging and underlines the need to scale up priority of ANC health education.

P.2.3.009 (A)

Born before arrival: user and provider perspectives on home deliveries and barriers to health facility childbirth in Kapiri Mposhi, Zambia

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Background Maternal mortality remains high in sub-Saharan Africa. Health facility intra-partum strategies with skilled birth attendance have been shown to be most effective to address maternal mortality. In Zambia, the health policy for pregnant women is to have facility childbirth. However, less than of the women utilize the facilities for delivery. 'Born before arrival' is used to describe childbirth outside a health facility. We aimed to explore how users and providers perceive the low utilization of health facilities at the time of childbirth.
Methods A qualitative study was conducted in Kapiri Mposhi, Zambia. Focus group discussions comprising women attending ante-natal clinic were conducted in 2007 as part of Response to Accountable priority setting and Trust in health systems (REACT) project. In-depth interviews were conducted in 2011 with 25 participants. Those interviewed comprised women who delivered at home, their husbands, and key informants. Information was collected on perceptions and experience of home and health facility childbirth, and reasons for delivery at home. Data was analysed by thematic content analysis.
Results Decisions to seek health facility care for childbirth were characterized by pull and push factors. Pull factors were safety of facility births, availability of drugs, privacy and confidentiality. Push factors were lack of respect by providers, not receiving prompt attention in labour, inadequate information received during childbirth; physical inaccessibility; cost of preparing for delivery; and cultural factors. The factors reflected the perceived quality of care by the community.
Conclusion The community in the study was aware of risks related to childbirth and had their perception of good quality care. Interventions that are accountable to the community could assist to improve quality of services and utilization in order to accelerate efforts to achieve MDG5.
Key words home deliveries, health facility childbirth, skilled birth attendance.

P.2.3.011 (A)

Utilization and quality of postpartum services for mother and infant in Kaya health district (Burkina Faso)

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Background Reducing maternal and child mortality and morbidity remains a worldwide challenge and particularly in developing countries. In Burkina Faso, maternal mortality rate is high. Our study aims to assess the utilization and the quality of postpartum services for mother and infant in Kaya district.
Methods From December 2012 to January 2013 we conducted a cross sectional study in 810 households in Kaya demographic surveillance site. Eight hundred and ten mothers who were in their first year postpartum have been interviewed. We conducted descriptive and multi-varied analysis of the collected data using STATA.
Results Preliminary results shows that 45% of mothers received a postpartum visit to the health facility: most visits took place on the first day postpartum (46%) followed by the visit at 6–10 days (40%). Monthly preventive and immunization visits of infants show a high utilization rate with 47% of the newborns receiving BCG vaccination during the first week, 66% and 64% receiving DTP (tetanus, diphtheria, polio, pertussis, B-haemophilus) at month 1 and month 2 after childbirth; and 74% at month 3–5. Results show that health workers enquire about the mother's general health status postpartum without asking specific questions about fever, general pain, and/or bleeding. Mother breast examination, check for infant anemia and cord inspection are done sometimes but with low percentage. Postpartum health care is not patient-centered; there is no real interaction between health workers and mother or infant.
Conclusion The study gives evidence on the low utilization and low quality of postpartum services. An intervention aiming to integrate maternal and infant postpartum services could increase the postpartum services in quality and coverage terms.

P.2.3.013 (A)

Review of maternal, neonatal and child health integrated services in the southern four provinces in Lao PDR

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Introduction The government of Lao PDR developed the Maternal Neonatal and Child Health (MNCH) integrated package strategy 2009–2015. In 2012, we carried out a situation analysis of MNCH services in the southern provinces (total population is around 1.2 million) to evaluate to what extent the service integration had been realized.
Methods Quantitative method with self-administered questionnaire was used to measure the service provision at all health facilities in the four provinces (four provincial hospitals, 26 district hospitals and 159 health centers).
Results In some health centers, basic materials, medicines and vaccines are out of stock. Furthermore, many health centers do not have basic/essential equipment and medicines for life-saving interventions (ex: oxytocin; 55%, magnesium sulfate; 13%, diazepam; 34%, bag and mask for newborn babies; 9%). The labor force at health center level is still limited. Moreover 30% of the staff members are not directly employed by the government. Number and proportion of health personnel who completed MNCH core trainings has imbalance among districts. Regarding outreach activities, 57% of all health centers have all main components (five vaccines, vermicides and vitamin A, iron and folic acid and four items of family planning). 12% of all health centers have certain deficit of vaccines for outreach. Basic vaccines are not available four times higher (24% vs. 6%) at the health centers with two or less personnel. This deficit is concentrated only in eight districts among 26. To reiterate, main courses of deficit of vaccines would be less number of the labor force in a health center and/or inappropriate management at district health office/health center.
Conclusion To provide MNCH services at health centers, we should consider the balance equity and integration in the resource-limited setting. Integrated package with more services might widen the gap between rich and poor, urban and rural areas in Lao PDR.

2.3.1 Obstetric Fistula; the incurable cases-what is the solution?

P.2.3.1.001 (A)

Patients' experience of obstetric fistula care in Mali and Niger

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Introduction Quality of life and psychological scales have highlighted social distress of obstetric fistula patients before and sometimes after surgery – whether continence was gained or not. Yet, few data are available on patients' real-life experience within a fistula repair centre. In a constructivist and bottom-up positioning, we consider patients' point of view as a prerequisite of care improvement. Accordingly, our research aims to gain insight of fistula patients' care perceptions.

Material and Methods During an 18 month mixed methods research in Mali and Niger, we conducted a total of 67 semi-directive interviews (1–7 interviews/woman) with 35 obstetric fistula patients and former patients at different stage of their care process and up to 5 years after it. Qualitative data were explored within a grounded theory approach and using phenomenological analysis. We focused on patients' perception of care before, during, after and beside surgery. Duration of care process emerged as core dimensions of our analysis.

Results Most of our participants suffered from fistula since more than 1 year (27/35) and had been operated on more than twice (18/35). Half of them were continent at baseline (9) or at the end of our research (7). Patients defined care as a process starting with first admission to a fistula repair centre and ending with continence gain or diagnosis of incurability. They rarely felt included in their own care process. Despite fragmented information, women understood pre- and post-op procedures quite well while surgery remained more obscure; especially in case of repeated unsuccessful attempts. Main cause of frustration was waiting period before surgery. Long-term stay in fistula repair centre was perceived as a double-edged experience: source of both comfort and stigma.

Conclusions Insight of obstetric fistula care experience highlights the daily struggle of long-term patients and the need of customized care.

P.2.3.1.002 (A)

Equity gap in utilisation of emergency obstetric care service in Cambodia, from 2007 to 2009

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Introduction and Objective Cambodia has recently diminished its maternal mortality ratio (MMR) rapidly: from 472 (DHS in year 2005) to 206 (DHS in year 2010). Possible explanations for this achievement are improvement of road network, increase of government budget to health sector which promotes to allocate midwives in health centers provides incentives both to health staff and pregnant women. Increase in provision of emergency obstetric care (EmOC) could be one of the factors to improve maternal health. However the magnitude of its contribution to decline MMR in Cambodia is unknown. We hereby report recent trend of utilisation of EmOC services in selected provinces in Cambodia from year 2007 to 2010.

Material and Methods The target areas of this study were Phnom Penh city (capital) and five provinces (Kandal, Kampong Cham, Kampong Chhnang, Kampong Speu, Kampong Thom). We have applied 'unmet obstetric need (UON)' indicator proposed by De Brouwere to measure the utilisation. Data on major obstetric interventions (MOIs: caesarean section and hysterectomy for haemostasis of postpartum haemorrage) is collected in all public hospitals and private clinics in the target area. The data were classified by areas of residence and indications of intervention.

Results and Conclusion We have confirmed an increasing trend in the rates of MOIs for absolute maternal indications (AMIs: placenta praevia, abruptio placentae, abnormal presentations, uterine rupture, severe postpartum haemorrage), which were 0.63%, 0.75%, and 0.85% in year 2007, 2008 and 2009, respectively. Although the same trend is observed in every province, there are substantial variations in the rates of MOIs for AMIs among the areas. Further effort to reduce this equity gap should be put, and this UON method is useful to monitor the trend.

Table 1 Estimated number of deliveries, total number of major obstetric interventions and major obstetric interventions for absolute maternal indications in some provinces in Cambodia, year 2007 to 2009

Area	Year	Estimated number of deliveries	Total number of MOIs	MOIs for AMIs	
				[*n*]	(%)
Total	2007	70,046	2866	438	0.63
	2008	72,532	4056	542	0.75
	2009	74,831	5685	638	0.85
Phnom Penh (capital)	2007	8789	1656	173	1.97
	2008	9393	2153	163	1.74
	2009	9893	3180	225	2.27
Kandal	2007	14,202	325	38	0.27
	2008	14,833	515	58	0.39
	2009	15,384	693	66	0.43
Kampong Cham	2007	20,567	533	122	0.59
	2008	21,043	865	184	0.87
	2009	21,553	1134	192	0.89
Kampong Chhnang	2007	7589	172	71	0.94
	2008	7856	207	56	0.71
	2009	8111	229	59	0.73
Kampong Speu	2007	8861	85	20	0.23
	2008	9173	197	61	0.66
	2009	9431	231	60	0.64
Kampong Thom	2007	10,038	95	14	0.14
	2008	10,234	119	20	0.20
	2009	10,459	218	36	0.34

P.2.3.1.003 (A)

Deemed incurable obstetric fistula patients' care pathways in Mali and Niger: a mixed methods research

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Introduction For some obstetric fistula patients, repair is not limited to one single short-lived care episode; it does lasts months or years. Despite surgery successes and progress, not all women gain continence nor have the opportunity to resume their former life. Care pathways of deemed incurable patients is to be considered in care improvement initiatives.

Material and Methods During an 18 month mixed methods research in five fistula repair centres within general hospital/maternity in Mali and Niger, we conducted interviews by questionnaire with 120 patients at three points of time and 67 semi-directive interviews with 35 patients and former patients at different stage of their care process and up to 5 years after it. We compared socio-obstetrical profile of deemed incurables (≥2 unsuccessful surgeries) with potentially repairable patients (≤1 surgery). Distribution of deemed incurables in four patients' mobility patterns during care process (homebound, itinerant, institutionalised and urbanised) was described and explored in-depth through case studies.
Results Our 109 patients' cohort counted 66 deemed incurables; 28 among them had been operated on five times or more. Fourty of the 43 potentially repairable patients gained continence during study. There was no difference in age, marital status and gravidity between two groups. Unsurprisingly, fistula duration, global fistula care process duration and aggregated time spend in fistula repair centres were significantly higher in the deemed incurables'group. Deemed incurables were present in the four mobility patterns. Place of stay during care process depends of intertwined factors related to patients medico-social and economical profile as well as health care organisation. Decision to settle in repair centre or community was rarely in patients' hands.
Conclusion Different care pathways plead for implementation of customized care to address specific needs of potentially curable and deemed incurable patients.

P.2.3.1.004 (A)

Living in obstetric fistula repair centres within general hospitals in Mali and Niger

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Introduction Different models of delivering fistula care co-exist throughout Africa. Each model has respective advantages and shortcomings in terms of holistic care provision and Health System organisation. Fistula patients usually live several weeks or months in repair centres. Some deemed incurable patients sometimes settle within repair centres or in their neighbourhoods. Hypothesizing that type of fistula repair centres might influence patients' further medico-social prospects, we explored daily life experience of patients in repair centres within general hospitals.
Material and Methods During an 18 month mixed methods research in five fistula repair centres within general hospitals/maternities in Mali and Niger, we conducted interviews by questionnaire with 120 patients at three points of time and 67 semi-directive interviews with 35 patients and former patients at different stage of their care process and up to 5 years after it. Our intensive fieldwork in repair centres was the opportunity to complete our data by direct observations. Starting from topographic observation, we analysed patients' perception of their life in fistula repair centres. We highlighted mechanisms of extended stay and ensuing consequences for patients as well as for Health System.
Results Because they are recent addition and/or because of their inpatients medico-social specificities, fistula repair centres within general hospitals have a location that is both protecting and ostracizing. Likewise, efforts to provide comfort or develop income generating activities also are double-edged. Need of physical rest conflicts with need to work for food, medicines or extras. Gaining an income within or outside the hospital contribute to women's empowerment but boomerang in encouraging institutionalisation. Sisterhood impact on women's peace of mind is often limited in time.
Conclusion Fistula repair centre daily routine is part of a complex set of factors that impact obstetric fistula patients' medico-social prospects – especially in case of extended or repeated stay.

2.5 Global burden of perinatal morbidity and mortality

P.2.5.001 (B)

Kangaroo mother care: achievements and challenges in a rural tanzanian hospital

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Introduction While infant and U5-mortality reduction is almost in line with MDG4, neonatal mortality keeps being high in Tanzania. Prematurity is one of the leading causes to neonatal mortality. The setting is a district referral hospital in rural Tanzania whose staff has attended a national training on Kangaroo Mother Care method. A subsequent external quality assessment performed on the hospital on August 2012 remarked unsatisfactory utilisation of KMC practice.
Materials and Methods This pilot study, preliminary to a 3 years project aiming at reducing neonatal mortality in the district, took place from December 15th, 2012 to April 30th, 2013. An expatriate neonatologist made sure that KMC practice was actually implemented on preterm newborns. For each newborn treated with KMC, the following data were collected:
1 Gestational age;
2 Weekly body weight;
3 Major complications.
Results Nine newborns have been enrolled in the study. Initial weight ranged from 800 to 1500 g. Eight out of nine enrolled newborns survived. The dead case is a baby exposed to HIV and with gestational age below 24 weeks. This is the only case that showed clinical signs of infection. One newborn encountered growth problems solved with temporary nutritional supplementation.
Conclusions Kangaroo Mother Care seems an affordable and sustainable strategy in a context with limited resources. It proves to be safe in terms of infections and other complications, even in very low birth weight newborns that would be considered at serious risk in a developed country. Although almost no material cost is required, mothers and nursing staff have to be keen, motivated and convinced of the effectiveness of the strategy. This is a challenge in our context. At the moment, the number of cases is too limited for any statistical analysis. However, KMC will be further implemented to reduce neonatal mortality at district level.

P.2.5.002 (B)

Blood stream infections among neonates admitted to a Vietnamese paediatric hospital

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INTRODUCTION Blood stream infections (BSI) causing septicaemia are among the major causes of neonatal morbidity and mortality globally, but have not been published from Vietnam previously. We describe positive blood cultures (BSI) and septicaemia related death among hospitalized neonates in Vietnam.
MATERIAL AND METHODS Among all neonates admitted to a tertiary paediatric hospital in South Vietnam in a 12 month period in 2009–2010, data on positive blood cultures were obtained. For neonates with positive culture, data were retrieved on isolate, antibiotics susceptibility, basic demography and clinics. Each neonatal death occurring was audited to determine septicaemia relation.
RESULTS Among 5802 neonates admitted, 2220 blood cultures were performed, of which 399 were positive (18%). Among these, 3% had central venous catheters, 16% were early onset (¡Ü3 days old), and 62/64 deaths were septicaemia related. Among BSI, 66% were known pathogenic and the remainder potential pathogenic. Staphylococcus Coagulase Negative. Gram-negative bacteria accounted for almost half, *Klebsiella* spp (n = 78), *Acinetobacter* spp (n = 58) and *E. coli* (n = 21) were the most frequent. Only three *Streptococus* spp were isolated. Further, antibiotic resistance was common, including resistance towards antibiotics empirically applied in the hospital.
CONCLUSION Among BSI in hospitalized Vietnamese neonates, the majority was known pathogenic and Gram-negative. No Streptococus group B were identified, in contrast to developed countries. The vast majority of deaths occurring were septicaemia related. Further, antibiotics resistance was common towards the antibiotics empirically used. Systematic surveillance should be considered to guide future BSI prevention and management.

Table 1 Distribution of 399 blood stream infections (BSI) among all 385 neonates and 62 neonates dying in relation to septicemia 14 duplet BSI culture samples with different organisms isolated at different times

Pathogenicity	Isolate	All BSI	Deaths
Known	Klebsiella spp	78	19
	Acinetobacter spp	58	10
	Escherichia coli	21	5
	Other Gram-neative bacteriae	35	8
	Streptococus spp	3	1
	Other Gram-positive bacteriae	16	3
	Candida spp	13	3
Potential	Staphylococcus coagulase negative	175	13
Total		399	62

Other Gram-negative bacteriae: spp *Enterobacter, Morganella, Pseudomonas, Proteus* and *Burkholderia.*

Other Gram-positive bacteriae: *Staphylococcus aureus* and *Enterococcus* spp.

P.2.5.006 (B)

Determinants and causes of neonatal mortality: a prospective population-based study in rural southern of Vietnam

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INTRODUCTION Although neonatal mortality (NM) remains a public health problem in Vietnam there is no published data on NM from community in southern of the country. The aim of our study was therefore to assess the neonatal mortality rate (NMR) and to identify causes and potential determinants of NM in Binh Thuan province.
METHODS We used Poisson 95% confidence intervals to study NM risk within a prospective population-based sample of infants born in all health facilities of Binh Thuan in the year 2010 (N = 13 803 live singleton births). The cause of deaths provided by health facilities was reviewed by a local pediatric physician.
RESULTS The overall NMR was 7.4 per 1000 live births (95% CI 6.0 C9.0 ⁄ 1000). Low birth weight/ pre-maturity (33.3%), birth asphyxia (22.5%), neonatal infection (21.6%), and congenital malformation (15.7%) were the major causes of neonatal death. Illiterate mothers (mortality ratio = 4.05; 95% CI 1.96–7.02), primigravidae (2.03; 95% CI 1.46–2.61), ethnic minority mothers (2.16; 95% CI 1.38–3.24), mothers living in rural area (1.99; 95% CI: 1.56–2.43), mothers aged <20 (2.00; 95% CI 1.12–3.30), or ¡Ý 35 (1.86; 95% CI 1.04–3.06) years old, prematurity (8.05; 95% CI 5.69–10.08), and low birth weight (18.51; 95% CI 13.61–23.99) were associated with neonatal mortality.
CONCLUSION These results suggest a high rate of NM in Binh Thuan. The programmes of health awareness should target mothers and babies at high risk.

Track 3: Non-communicable diseases

3.1 The global burden of diabetes – relevance in the developing world

P.3.1.001 (B)

A matter of mastering the game – an analysis of strategies for health among people with type 2 diabetes in Kampala, Uganda

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INTRODUCTION Type 2 diabetes poses immense challenges to both health systems and individual patients. In Uganda, patients are to a large extent dependent on their own strategies to successfully manage their diabetes. With Bourdieu's theoretical concepts of field, capital and habitus, this study explored strategies for health deployed by people living with type 2 diabetes in Kampala.

MATERIAL AND METHODS The data was collected from an out-patient diabetes clinic of a national referral hospital in Kampala from January to February 2012. The data comprises (i) Semi-structured interviews with 10 type 2 diabetes patients, (ii) two key informant interviews with health professionals dispensing diabetes care at the hospital and three diabetes specialists, (iii) 10 h of observations of cooking in patient interviewees' homes, and (iv) 30 h of observations in- and outside the diabetes clinic.
RESULTS Patients deploy a range of strategies to manage diabetes and improve health. To patients struggling with uncertain life conditions, strategies for health are focused on survival, which result in pragmatic short-term treatment goals. Social and economic capital of either the family or church is drawn upon to improve access to medicine and counseling. Finally, some patients deploy a strategy of appearing in control, whereby they position themselves as competent in controlling their health. This strategy offers better chances of claiming rights and making demands to the health system, but it is limited to patients with sufficient resources. Hence, the appropriateness of strategies – what is considered logic – differs between different habitual dispositions.
CONCLUSIONS By bringing structural life conditions of people into the centre of analysis, the study's emphasis is on practice, rather than behaviour. Individuals with diabetes in Kampala have unequal possibilities for managing illness, because of differences in resources, but also, because of unequal competences as strategic 'players' in the game of health.

P.3.1.002 (B)

Differences in illness perception of Type-2 diabetes between patients and their health care givers in Ghana

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INTRODUCTION Type-2 diabetes is a serious health concern in Ghana. It has been proposed that successful treatment of diabetes patients rely on coherence in illness perception between patients and their health care givers (HCGs). The aim of this study was to investigate illness perception of type-2 diabetes between patients and HCGs and to assess the patients believes about medicine and adherence to treatment. To our knowledge no study has investigated this in Ghana.
MATERIAL AND METHODS We conducted a cross-sectional questionnaire-based study in the city of Kumasi, Ghana, at two different hospitals. The questionnaires were translated into the Ghanaian dialect Twi for the patients. Questionnaires consisted of the 'revised illness-perception questionnaire' (IPQ-R). In addition patients completed the 'believes about medicine questionnaire' (BMQ) and the 'medical adherence report scale' (MARS). All patients were interviewed by two interviewers, while the HCGs completed the questionnaires themselves in English.
RESULTS We obtained 116 completed questionnaires from patients and 20 from HCGs. Significant differences in illness perception were found between HCGs and patients in 4 IPQ-R items. HCRs perceived diabetes as being less chronic ($P < 0.001$), as being more cyclical of nature ($P < 0.001$) and as having less personal control over their diabetes ($P = 0.011$) than perceived by the HCGs. Patients scored higher on 'illness coherence' than estimated by HCGs ($P = 0.002$). Regarding importance of risk-factors, HCRs scored significantly lower than HCGs ($P = 0.002$). A positive correlation between general believes in medicine and self-reported adherence (0.266; $P < 0.01$) was found.
CONCLUSION For the first time it has been shown that Ghanaian diabetes patients and their HCGs perceive type-2 diabetes in significant different ways. Holding different believes about type-2 diabetes might cause isunderstandings and finally compromise treatment of the patients through low adherence. More research is needed to elucidate the consequences of this incongruence in illness perceptions and ways to overcome it.

3.2. The double disease burden

3.2.1 The double burden of communicable and non-communicable diseases

P.3.2.1.002 (A)

Cross-sectional survey of renal function in HIV-positive and HIV-negative clients of the Lighthouse counselling and testing centre in Lilongwe, Malawi

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BACKGROUND Renal function of people living with HIV/AIDS (PLWHA) can be negatively affected through HIV-associated nephropathy, comorbidities like hypertension or diabetes and side-effects of ARVs such as Tenofovir. In Malawi, where HIV prevalence is 11.9%, little is known about the prevalence of non-communicable diseases and in particular of chronic kidney disease (CKD). This study set out to collect data on renal impairment in HIV-infected and -uninfected individuals in Lilongwe, Malawi.
METHODS A cross-sectional survey was conducted at an HIV-counselling and testing centre at the Lighthouse, Lilongwe. Between January and March 2012 we enrolled 363 ART-naïve

Renal function (eGFR in ml/min/1.73 m^2)	Total, $n = 363$ (%)	HIV infected, $n = 116$ (%)	HIV uninfected, $n = 247$ (%)
No inpairment: eGFR ≥60, – proteinuria	316 (87.0)	90 (77.6)	226 (91.5)
Kidney damage with normal or ↑GFR: eGFR ≥90 + proteinuria	20 (5.5)	8 (6.9)	12 (4.9)
Kidney damage with mild ↓ GFR: eGFR 60–89 + ;proteinuria	16 (4.4)	13 (11.2)	3 (1.2)
Moderate ↓ GFR: eGFR 30–59 ± proteinuria	9 (2.5)	5 (4.3)	4 (1.6)
Severe ↓ GFR: eGFR 15–29 ± proteinuria	2 (0.6)	–	2 (0.8)

HIV-infected and -uninfected individuals >18 years, measured blood pressure and BMI and asked about other risk factors for renal impairment. Serum samples were analyzed for Creatinine and Cystatin-C and urine for Albumin/Creatinine Ratio (ACR). Estimated glomerular filtration rate (eGFR) was calculated by using the Cystatin-C equation developed by van Deventer *et al.* (2011). Proteinuria was defined as ACR >3 mg/mmol Creatinine. Proportions of renal impairment were compared between HIVinfected and -uninfected individuals.
Results Of 363 individuals (48% female) 116 (32%) were HIV-infected and 247 (68%) were not. Age (median 31, IQR 26–39) and gender were similar between both groups but BMI was lower in HIV-infected individuals 20.8 (19.0–22.9) vs. 22.6 (20.9-26.2). Renal impairment was present in 13% of participants and 3.1% had moderate to severe dysfunction, none of whom had previously been diagnosed with chronic kidney disease. Prevalence of renal dysfunction was higher in HIV-infected individuals. The results of renal impairment are grouped according to thresholds for stages 0–4 in CKD.
Conclusion Assessment of renal function in countries like Malawi warrants more attention in particular for PLWHA and in regard to the introduction of Tenofovir in first-line ART regimen.

P.3.2.1.003 (A)
Tuberculosis and diabetes care in Karnataka, India; the perspective of patients and health care providers

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Introduction Diabetes (DM) triples the risk of tuberculosis (TB). Approximately 61 million people have DM in India, and each year 2–2.5 million develop TB. This double burden challenges the overburdened health system. Professional and institutional divide persists between communicable and non-communicable diseases in spite of evidence of their interactions. This study identifies challenges and opportunities for an effective response to TB/DM through the perspectives of patients and health care providers (HCP).
Methods An ethnographic field study was conducted May–July-2012, Karnataka. Twenty-eight men and women with TB/DM, and 10 private and government HCP from allopathic and indigenous medical systems, were interviewed, and participant observations conducted in private and government facilities.
Results Both patients and HCP struggle managing TB/DM co-morbidity, as their aetiologies and management approaches appear antagonistic, and the double burden manifests itself as more than double. The challenges are largely determined by the health system's inability to address co-morbidities not falling under the same medical specialities. The many health care options are not expressed in equal access- particularly evident for DM, which largely depends on availability, affordability and acceptability of services. Fragmented service delivery is a major challenge, giving rise to contradictory treatment advice for TB and DM and possibilities for care. Non-adherence and frequent change of HCP may seem irrational and dangerous to patients' health, but are sensible if considered as individual strategies to manoeuvre in the uncertainties created by TB/DM.
Conclusions There is a need for integrating TB/DM services through primary care, acting as single point of entry for diagnosis, treatment and monitoring of TB/DM and other chronic diseases, as well as referral site for specialised treatment. However, integrating TB/DM services is not immediately acceptable to all patients, who prefer specialised care, or to HCP; and their perspectives need to be assessed if integration is to be successful.

P.3.2.1.004 (A)
Physical activiy and overweight among adolescents in Ho Chi Minh City, Vietnam

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Introduction Ho Chi Minh City, a largest city of Vietnam, has a nutrition transition leading to increase rapidly in the prevalence of overweight/obesity among adolescents whose lifestyles are becoming more sedentary with lower levels of physical activity (PA). Adolescence obesity increases the risk of cardiovascular- and chronic diseases in adulthood. Our study aimed to assess time spent in moderate to vigorous physical activity and its relationship with body mass index (BMI) among adolescents of Ho Chi Minh City (HCMC) in Vietnam.
Material and Methods In a cross-sectional study conducted in HCMC in 2010, 2024 junior high school students were measured for BMI and questioned on their PA, based on validated PA questionnaire. Level of activity was classified as physical active or inactive using standard criteria. Overweight and obesity definition was based on the age- and sex-related Internal Obesity Task Force BMI cutoff values.
Results Adolescents spent 1.5 h/day (median) in PA, with higher values for boys (124 min/day, IQR [69 212] min/day) than girls (67 min/day, IQR [35 127] min/day) ($P < 0.001$), and 33.9% were categorized as inactive. Children living in richest family spent significantly higher time in PA than the others ($P < 0.001$). Prevalence of overweight/obesity was 21.1%. Among children aged 13–14 years, overweight/obesity boys spent less time in moderate to vigorous PA ($P > 0.18$), while overweight/obesity girls spent more time in moderate to vigorous PA ($P < 0.02$).The percentage of inactive tended to increase across BMI category among boys ($P < 0.04$) but the trend decreased among girls ($P < 0.04$).
Conclusion In HCMC, there is a considerable percentage of adolescents who are inactive. Boys aged 13–14 years who are overweight/obesity spend less time in PA and have a higher percentage of inactive than others. Public health intervention programs are needed to reduce inactive time among adolescents, especially in boys aged 13–14 years.

3.3 Malnutrition (malnutrition and infection)

3.3.1 Acute malnutrition: short and long-term consequences

P.3.3.1.001 (A)
Moderate and severe acute malnutrition attending at rural hospital in Southern Ethiopia during 2 years (September 2010–August 2012)

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Introduction Moderate acute malnutrition (MAM) and severe acute malnutrition (SAM) are associated with high rates of mortality and morbidity and requiring specialised treatment and prevention interventions. The objective of the present study

was to evaluate cases of MAM and SAM during 24 months to Outpatient department (OPD) in a rural hospital in Southern Ethiopia.
MATERIAL AND METHODS The Gambo General Hospital (GGH) is a 150-bed rural general hospital. Most of the population live in a rural setting. We prospectively recorded patients from 6 months to 5 years old with MAM or SAM attending in OPD from September 2010 to August 2012.
RESULTS During period of study 10.036 children were attended in OPD and were screened for malnutrition. Of them 806 (8.0%) children was malnourishes: 630 (78.2%) with SAM and 176 (21.8%) with MAM. 53.6% were male. Children <2 years constituted for 58.3% of children attended. SAM were less common in children <2 years (70.4%) than in >2 year (89.0%)($P < 0.001$). An initial diagnosis of diarrhea or respiratory tract infection were more common in children <2 years (54% and 25.1%) than in >2 year (26.6% and 10.1%) ($P < 0.001$). On the other hand, the initial diagnosis of malnutrition syndrome was less frequent in children <2 years (15.1%) than in >2 year (57.6%) ($P < 0.001$). Of 630 cases of SAM, oedema was less frequent in children <2 years (20.2%) than in >2 year (60.6%) ($P < 0.001$). However, oedema +3 were more frequent in children <2 years (42.6%) than in >2 year (4.7%) ($P < 0.001$). The children with MAM used to consult to OPD for diarrhea or respiratory tract infection (99.6%) ($P < 0.001$) and children with SAM for malnutrition (56.7%) and less for other diseases (43.3%) ($P < 0.001$).
CONCLUSIONS Malnutrition is endemic in our area and profile of malnutrition depending of age of patients.

Table 1 Comparison profile of malnutrition depending of age of patients (<2 years old vs. ≥2 years)

	Total	<2 year $n = 469$	≥2 year $n = 335$	P
Type of malnutrition				
MAM*	176 (21.8)	139 (29.6)	37 (11.0)	<0.001
SAM†	630 (78.2)	331 (70.4)	299 (89.0)	
Sex				
Female	374 (46.4)	218 (46.4)	156 (46.4)	0.8
Male	432 (53.6)	252 (53.6)	180 (22.3)	
Year				
September 2010–August 2011	433 (53.9)	271 (57.8)	162 (48.4)	0.008
September 2011–August 2012	371 (46.1)	198 (42.2)	173 (51.6)	
Diagnostic				
Diarrhoea				<0.001
Yes	343 (42.6)	254 (54.0)	89 (26.6)	
No	435 (55.9)	198 (43.8)	237 (72.7)	
Respiratory tract infection				
Yes	152 (18.9)	118 (25.1)	34 (10.1)	<0.001
No	626 (80.5)	334 (73.9)	292 (89.6)	
Malnutrition diagnosis				
Yes	264 (32.8)	71 (15.1)	193 (57.6)	<0.001
No	514 (66.1)	381 (84.3)	133 (40.8)	
Oedema, only SAM ($n = 629$)				
No	355 (56.4)	264 (80)	91 (30.4)	<0.001
Yes	275 (43.7)	67 (20.2)	208 (69.6)	
1+	41 (6.5)	21 (6.4)	20 (6.7)	
2+	68 (10.8)	23 (7.0)	45 (15.1)	
3+	165 (20.5)	143 (42.6)	22 (4.7)	

*Moderate acute malnutrition.
†Severe acute manlnutrition.

Table 2 Comparison profile moderate acute malnutrition (MAM) versus severe acute manlnutrition (SAM)

	Total	MAM ($n = 176$)	SAM ($n = 630$)	P
Age				
<2 year	470 (58.3)	139 (79.0)	331 (52.5)	<0.001
>2 year	336 (41.7)	37 (21.0)	229 (47.5)	
Sex				
Female	374 (46.4)	82 (46.6)	292 (46.3)	0.9
Male	432 (53.6)	94 (53.4)	338 (53.7)	
Year				
September 2010–August 2011	433 (53.9)	109 (61.9)	324 (51.6)	0.015
September 2011–August 2012	371 (46.1)	67 (38.1)	304 (48.4)	
Diagnostic				
Diarrhoea				
Yes	343 (42.6)	115 (67.3)	228 (37.6)	<0.001
No	435 (55.9)	56 (32.7)	379 (62.4)	
Respiratory tract infection				
Yes	152 (18.9)	51 (29.8)	101 (16.6)	<0.001
No	626 (80.5)	120 (70.2)	506 (83.4)	
Malnutrition diagnosis				
Yes	264 (32.8)	1 (0.6%)	263 (43.3)	<0.001
No	514 (66.1)	170 (99.4)	334 (56.7)	
Oedema, only SAM ($n = 629$)				
No	355 (56.4)		355 (56.4)	
Yes	275 (43.7)		275 (43.7)	
1+	41 (6.5)		41 (6.5)	
2+	68 (10.8)		68 (10.8)	

P.3.3.1.002 (A)

Intestinal parasites in admitted children aged 0 to 59 months, with servere acute malnutrition, in Central Hospital of Nampula, Mozambique

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Intestinal parasites infections and malnutrition, especially in developing countries, are an important Child Health problem, as it compromises the child's development. Despite its importance few hospital-based studies have been conducted. The present work aimed at determining the frequency of intestinal parasites

in children from 0 up to 59 months of age, and the relation with severe acute malnutrition (SAM) at the Malnutrition ward, Department of Pediatrics at the Central Hospital of Nampula, Mozambique.
Material and methods From March to May 2011 we studied 189 children with SAM (zscore weight for height <−3, mid-upper arm circumference <115 mm and/or oedema). Microscopic analysis was performed on a sample of stool for detection of intestinal parasites. The relation between intestinal parasites infection and SAM was analysed using statistical methods.
Results Of the 189 children, 50.3% were males and 49.7% were females, the mean and median age was 19 and 18 months. 49.2% (93/189) of children had Maramus, 29.6% (56/189) Kwashiorkor and 21.2% (40/189) Kwashiorkor Marasmatic. Children with oedema presented a median age (22 months) higher than median of children without oedema (15.54 months) ($P < 0.001$). The parasitological examination of feces identified that 22.8% children were infected with pathogenic intestinal parasites. The most frequent parasites were Giardia duodenalis and Strongyloides stercoralis in 13.2% and 6.4% of children. The frequency of pathogenic intestinal parasites was higher in children between 24 and 48 months.
Conclusions In this study no association was found between infection by intestinal parasites and clinical forms of SAM. However, the results showed that the clinical form of SAM with oedema and the infection by pathogenic intestinal parasites were more frequent in children between 24 and 48 months of age. Despite the small sample, this study reinforces the importance of the diagnostic of intestinal parasites in children with SAM for their better clinical management.

P.3.3.1.003 (A)

Analysis of anthropometric indicators of malnutrtition in chldren from São Tome Africa: a change of pattern?

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The undernutrition is a multifactorial and dynamic process with different patterns according to changing health and socio-economic conditions. for global undernutrition in children under 5, it is described a trend to decrease in both, prevalence and severity in the last decade. This situation shifts the curve to mild forms of undernutrition, a public health concern in developing countries, due to its high prevalence and elevated risk of mortality. The objective of this study was to analyse the anthropometric indicators of malnutrition in a group of children from São Tomé e Principe (STP), Africa and to establish the severity pattern including mild forms.
Materials and Methods A cross sectional study was conducted in six districts for STP, from November to December in 2011. Demographic data were registered and weight and height were measured. Z scores were calculated for weight for age (WAZ), height for age (HAZ), weight for height (WHZ) and body Mass Index for age (BMIZ), using the WHO Anthro and WHO Anthro Plus software v 3.2.2. Each anthropometric indicator was evaluated according to severity (z score <−3, −2 to −3, −1 to −2, −1 to >0). Statistical analysis was performed using SPSS v21 statistical software.
Results A total of 417 children were included in the study, with mean of age 63.43 months and an equitable distribution by gender. 64.5% of the children were >60 mo. A 6.71% of stunting (0.5% severe and 6.2% moderate), 5.03% of underweight (0.2% severe and 4.8% moderate) and 3.83% of wasting (0.7% severe and 3.1% moderate) were detected. Mild malnutrition ranged from 15 to 23% for the three anthropometric indicators.
Conclusions This study showed a change of undernutrition pattern, with an increase in mild forms. These mild malnourished children should bring new challenges in their aproach, managament and intervention.

P.3.3.1.004 (A)

Anti-nutritional compounds in food aid products used for prevention and treatment of malnutrition in infants and young children

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Introduction Anti-nutrients are found in most plant foods, especially unrefined cereals and legumes. The contents of anti-nutrients in processed foods depend on the mechanical and thermal processing. Anti-nutrients in complementary foods can make nutrients inaccessible for absorption in the gastrointestinal tract, by forming insoluble complexes or by inhibit enzymatic digestive processes. The aim of this study was to screen complementary foods from developing countries for selected anti-nutritional compounds.
Material and Methods A total of 36 products representing foods used in food aid programmes (blended flours and Ready-to-Use-Foods (RUTFs), local blended foods, fortified instant porridges and 'baby foods' were sampled. Whole grain instant cereals available on the European market were included as a 'worst case' scenario for anti-nutrient exposure in Europe. Samples were analysed for inositol phosphates, with 4-6 phosphategroups (IP4-IP6); polyphenols and inhibitors of trypsin and chymotrypsin. Contents of minerals (iron, zinc and calcium) in which absorption or utilization is affected by anti-nutrients were analysed.
Results and Conclusions The phytate content ranged from 68 to 1536 mg/100 g, confirming a persistent problem of high levels of phytate in processed cereal and legume based products. The phytate:Fe molar ratio exceeded the recommended level of <1.0 in 32 of the 36 products, while 25 products met the recommended molar ratio for phytate:zinc of 15. The total polypenols varied from 1.3 to 9.3 mg gentisic acid equivalents/g. Low-weight soluble polyphenols are nutritionally more relevant to analyse for than total polyphenolic compounds. Trypsin and chymotrypsin inhibitors and lectins were found in residual amounts in most products indicating efficient degradation by heat processing. However, young infants and malnourished children may have reduced pancreatic function, and upper limits for residual trypsin inhibitors are needed.

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P.3.3.1.005 (A)

Serum phosphate and magnesium levels in children recovering from severe acute undernutrition

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Background Refeeding of children with severe acute malnutrition (SAM) increases the need of phosphorus and magnesium. As these children are likely to be depleted in phosphorus and magnesium there is a risk of developing refeeding syndrome during the initial days of treatment. Furthermore, there is a need for phosphorus and magnesium for replenishment of tissue deficits and catch up growth during the rehabilitation phase. Phosphorus and magnesium are believed to be the limiting nutrient in F-75 and F-100, respectively. Phosphorus is added to the commercial formula of F-75 to address this problem but the bioavailability is unknown and the effects have not been assessed.
Objective To describe serum phosphate and magnesium levels during rehabilitation with F-75 and F-100.
Subjects and Methods Prospective study with repeated blood sampling at admission, start on rehabilitation phase and discharge in SAM children age 6–60 months admitted to Jimma Hospital, Ethiopia.
Results Seventy-two children were enrolled. Oedema was present in 50 (69%) and mean ± SD age was 35 ± 14 and 26 ± 12 months for children with and without oedema, respectively. Serum phosphate was low at admission (0.92 ± 0.34 mM), whereas magnesium was within the normal range (0.95 ± 0.23 mM). Both increased with clinical improvement and at the time of discharge mean phosphate and magnesium was 1.38 ± 0.28 mM and 1.13 ± 0.17 mM, respectively. No difference was seen between children with and without oedema in phosphate and magnesium.
Conclusion The low phosphate level at admission clearly indicated that these children were deficient in phosphorus and there was a need for replenishment. Phosphate increased during admission but was not normalized upon discharge. This possess a potential problem for the further recovery of the child as additional phosphate is needed for catch-up growth and local diets are likely to be low in bioavailable phosphorus. The content and bioavailability of phosphorous in F-75 and F-100 should be reconsidered.

P.3.3.1.007 (A)

Stunting, overweight in young children occur simultaneously in rural South Africa, and are related to quality of diet

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Background Stunting remains a concern in developing countries, especially in rural areas, and has been linked to nutritional deficiency, food insecurity and a wide range socio-economic factors. Overweight and obesity, also in combination with stunting, now rival under-nutrition and linear growth retardation as worldwide public health concerns for young children.
Objectives The aim of this study is to quantify malnutrition in children 1–5 and to determine risk factors and associations between dietary intake and health status.
Methods Simple random sampling was used to select 480 child participants from the Dikgale HDSS database. Anthropometric measure was assessed and food intake data was collected using a 24-h recall survey.
Results Of the 206 children studied anthropometrically 37.1% are stunted, 20.6% are overweight, 10.2% are stunted and overweight, 4.9% are underweight, 4.5% are wasted according to WHO standards. Stunted children consumed significantly less vitamin A than non-stunted children, both HAZ and WHZ scores are significantly related to vitamin A intake in regression analysis. Food variety scores are lower in children who are stunted and overweight than in normal children, and 17% of the population had very low dietary diversity scores.
Conclusions Prevalence stunting and overweight are most prominent in children 1–3 years old and are related to quality of diet. Consumption of vitamin A rich foods, particularly carotenoids from fruits and vegetables, during this developmental window are protective against stunting. This study highlights the increased prevalence of overweight and obesity in young children in rural areas of Africa and the need for interventions focusing on consumption of vitamin A rich fruits and vegetables and raise awareness about growth monitoring, and dangers of childhood overweight.

3.3.2 Complementary feeding: prevention of malnutrition

P.3.3.2.001 (A)

Development of local processed complementary food products – 'WinFoods' – in Cambodia, for food aid programmes for prevention of child malnutrition

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Introduction Access to nutritionally adequate complementary foods is critical to prevent or reduce growth faltering during the critical complementary feeding period of age 6–24 months of age. In food insecure populations, food aid organizations [e.g. World Food Programme (WFP)] rely on imported standard products, in particular Corn-Soya-Blends (CSBs). WFP has over the past years recognised that availability of local products has multiple advantages. The aim of the WinFood project in Cambodia was to identify nutrient-dense local foods to be used in development of complementary food products, and test these in a randomized human intervention trial.
Materials and Methods Through a field survey, 84 traditional plant- and animal foods were identified. Selected foods were sampled and screened for iron and zinc contents. Some animal-source food – small fish species, some insects and an edible tarantula – were identified to have high iron or zinc contents.
Results and Conclusions Two processed rice-based complementary foods were developed based on iron- or zinc rich local food: (i) A 'WinFood' (WF) with two defined fish species, Esomus longimanus and Paralaubuca typus (12% dry weight (DW) fish), and an edible spider/tarantula, Haplopelma sp (2% DW); (ii) A 'WinFood-lite' (WF-L) version with mixed small fish species selected for low price (10% DW). Both products contained 4.5% soya-oil and 4.5% sugar, to adjust energy-density and taste. In addition, the WF-L product was fortified with mineral- and vitamin-mix similar to CSB products. The

grinded WinFood products were precooked by extrusion to a semi-instant 'babyfood' by a small local food manufacturer. Experimental batches of 2 tonnes per product for human intervention trial were produced (results reported elsewhere). The WinFood product development and production demonstrated that nutritious processed complementary foods can be developed and produced in Cambodia, thereby contributing to the prevention of malnutrition in infants and young children.

P.3.3.2.002 (A)

Prevalence of poor iron status among 6 months old infants in a rural part of Cambodia differs by infant feeding practice

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Introduction Concerns have been raised that exclusive breastfeeding (EBF) for six months may not be optimal with respect to iron status of infants. Pre-dominant breastfeeding (PDBF) and partially breastfeeding (PBF) of infants before the age of six months are common in Cambodia.
Objective To establish whether iron status differs among six months old infants fed on diverse infant feeding practices in a rural part of Cambodia.
Material and Methods Four hundred and four infants aged six +/− 1 months were enrolled between March and May 2011 in Prey-Veng province, Cambodia. Venous blood was obtained for haemoglobin (Hb) concentration (HemoCue®) and plasma ferritin and AGP concentrations (ELISA). A semi-structured questionnaire on background characteristics and infant feeding was administered to caretakers of 328 infants. Feeding practice of breastfed infants was classified according to WHO definitions.
Results Complete data were available for 309 breastfed infants (EBF $n = 50$, PDBF $n = 33$, PBF $n = 226$). There was no difference in mean Hb or prevalence of anaemia among the groups. In contrast, plasma ferritin was different between groups ($P < 0.05$) and lowest in PDBF and highest in PBF infants. The prevalence of ID also differed among feeding groups ($P < 0.01$), with 35.4% in EBF infants, 22.6% in PDBF infants and 13.7% in PBF infants, respectively. The prevalence of IDA was lowest in PBF (11.6%) and highest in EBF infants (25.0%), but the difference between groups was only marginally significant ($P = 0.050$).
Conclusions EBF practice could be a risk factor for ID/IDA in infancy, and more research in the role of EBF on iron status is warranted. Other risk factors not investigated in this study which could affect Hb concentration and iron status include the occurrence of haemoglobinopathies, deficiencies in other micronutrients, parasite infections, rapid growth and suboptimal iron stores at birth. Subsequent studies should implement further data for the control of possible confounding.

P.3.3.2.003 (A)

The use of linear programming retrospectively to clarify if an intervention product can optimize the complementary food diet – WinFood study from Cambodia

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Introduction A mathematical approach known as linear programming (LP) has in the resent years demonstrated as a tool

Table 1 Marker of inflammation and iron status by feeding practice in 309 infants[1]

	Exclusively breastfed[2] ($n = 50$)	Pre-dominantly breastfed[2] ($n = 33$)	Partially breastfed[2] ($n = 226$)	P-value[3]
Plasma Alpha 1-Acid Glycoprotein, g/l	0.62 (0.29)[PBF*]	0.66 (0.31)	0.78 (0.41)	0.014[a]
Elevated[4], *n* (%)	2 (4.0)	2 (6.1)	36 (16.0)	0.035[b]
Age[5], months	5.49 (0.30)[PBF#]	5.60 (0.35)[PBF§]	5.88 (0.47)	<0.001[a]
Male[5], *n* (%)	29 (60.4)	19 (61.3)	101 (53.2)	0.519[c]
Haemoglobin[5], g/l	107.8 (11.5)	106.2 (11.7)	108.0 (8.7)	0.708[d]
<110, *n* (%)	25 (52.1)	16 (51.6)	111 (58.4)	0.616[c]
Plasma ferritin[5,6], μg/l (IQR)	24.2 (10.0–48.7)	20.4 (12.0–34.5)[PBF**]	33.6 (17.6–58.9)	0.014[d]
<12, *n* (%)	17 (35.4)	7 (22.6)	26 (13.7)	0.002[c]
Iron deficency anaemia[5,7], *n* (%)	12 (25.0)	6 (19.4)	22 (11.6)	0.050[b]

[1]Breastfed subjects with complete set of parameters.
[2]Values are mean (SD), unless otherwise indicated.
[3]Significances of difference between groups were assessed with: [a]ANOVA *Post hoc* Tukey HSD, [b]Kruskal–Wallis test, [c]Pearsons chi-square test, [d]ANCOVA (covariate gender).
[4]Alpha 1-Acid Glycoprotein >1.2 g/l.
[5]Infants with elevated AGP excluded (EBF group $n = 48$, PDBF group $n = 31$, PBF group $n = 190$).
[6]Geometric mean (interquartile range).
[7]Iron deficiency anaemia: Hb < 110g/l and plasma ferritin <12 lg/l.
Significant difference to partially breastfed group in pairwise comparison: [PBF*]$P < 0.05$ (MD 0.16 g/l; 95% CI 0.02–0.30 g/l), [PBF#]$P < 0.001$ (MD 0.40 months; 95% CI 0.23–0.56 months), [PBF§]$P < 0.01$ (MD 0.28 months; 95% CI 0.09–0.48 months), [PBF**]$P < 0.05$ (MD 13.2 lg/l; 95% CI 2.3–29.5 lg/l).

to optimise the complementary food for children by generating population specific food-based nutrition recommendation. An efficacy trial was recently conducted, where two developed products [WinFood (WF) and WinFood Lite (WF-L)] were compared to standard Corn-Soy-Blend products (CSB+ and CSB++). The objective of the present study is to demonstrate how LP can be used retrospectively to clarify whether the WinFood and CSB products could optimize the complementary food diet of infants 6–11 months of age in Cambodia.
Material and Method Model parameters for a baseline diet, without any of the intervention products, were defined using dietary data collected in a cross-sectional survey of 6–11 months old infants (n = 78) living in Prey Veng Province, Cambodia. Afterwards, four intervention diets were defined, by including each intervention products one at the time into the baseline diet model.
Results The baseline diet could not theoretical achieve the recommended nutrient intake (RNI < 92%) of thiamine, riboflavin, niacin, folate, vitamin B-12, calcium, iron and zinc. None of the intervention diets could cover the theoretical nutrient gaps. CSB+ and CSB++ gave the highest achievable levels of RNI in iron (54% and 45% of RNI) and zinc (108% and 95% of RNI), but WF-L had a higher estimated bioavailable iron and zinc than CSB+. These results correspond to the results of the efficacy trial conducted with the four products.
Conclusion This study demonstrated how LP successfully can be used retrospectively in designing intervention products. None of the four intervention products succeed to cover the theoretical nutrient gaps for this Cambodian population, but with the higher estimated bioavailable zinc in WF-L and CSB++ clarify why these products were superior to support linear growth than WF and CSB+.

3.3.3 Nutrition and HIV interactions

P.3.3.3.001 (B)

Combating HIV/AIDS in rural communities through food and nutrition security: the role of women led micro-livestock enterprises and horticultural production in Agnrokopé Village (Togo)

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Introduction Togo 2010, AIDS prevalence 3.2%. Agriculture contributes 26% GDP, and 60% earnings. 2AVIE project. Forty percent of households in Agnrokopé village are involved in rural agriculture.
Scope of the Research Project The research project is being undertaken over a 3 year period, starting July 2007 in Agnrokopé village. According to the Togo Government Economic Survey of 2011, the population stood at 29 000 people. The study was undertaken within 11 out of the 15 wards in Agnrokopé village.
Population of the Study The target population for the baseline survey was HIV/AIDS affected households enrolled in the two major HIV/AIDS support groups in the village, namely: (Catholic dioceses of Agnrokopé village: Centre Marziano and 2AVIE).
Baseline Survey Households with a child aged between 10 and 15 years of age formed the sampling frame. Based on a census of all households with an index child purposive sampling was then used to select the households. Data was collected on livelihoods, and food and nutrition security.
Interventions Dairy Goats From the initial intervention group, 40 households will benefit from a dairy goat each, the payment is based on returning back to the project the first kid, so as to pass it to the another beneficiary.
Vegetables Participating households (80) were divided into two clusters, namely: those with their farming space, and those without.
Capacity Building Vegetable training; group leadership and dynamics; goat training.
Challenges The dependency syndrome amongst PLWHA's. Urban by-laws on Urban Agriculture. Attendance to farms due to sickness and distance to the farms.
Conclusions and Recommendation Systematic implementation of interventions after an initial baseline survey and participatory interaction. Partnerships in implementing projects. Skills training and presenting.

3.4 Mental health interventions in general health care and HIV care settings in low and middle income countries

P.3.4.001 (B)

Mental health research for the 2011 flood affected people in Thailand

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Introduction From the end of September 2011, Thailand experienced floods that affected 20 million people in 19 districts, killed more than 530 people, and damaged or destroyed more than 2 million homes and factories. We carried out a health impact assessment using questionnaire surveys to assess the prevalence of and risk factors for the psychosocial consequences of this flood in Thailand.
Material and Methods Surveys were conducted in two regions around Bangkok. Exposure variables included the presence of flood water in the home, evacuation and disruption to essential services, perceived impact of the flood on finances, house values. Validated tools were used to assess psychosocial outcome (mental health symptoms): K10 for depression and PTSD checklist short-form for post-traumatic stress disorder. Multivariable logistic regression was used to describe the association between water level in the home and psychological outcome adjusted for age, sex, occupation, presence of an existing medical condition.
Results Total 408 people answered. Flood affected people were higher both K10 and PTSD score than non-affected people (mean K10 score; 10.85 vs. 9.18, $P < 0.05$, mean PTSD score; 18.63 vs. 15.80, $P < 0.05$). Flood affected people were more likely to report depression (OR 2.20, 95% CI 1.27–3.83) and PTSD (OR 3.06, 95% CI 1.81–5.19). Disruption essential services especially electricity stop increased adverse psychological outcomes.
Conclusions The psychosocial and mental health impact of flood is a growing public health concern and improved strategies for minimizing disruption to essential services and financial worries need to be built in to emergency preparedness and response systems. Public health agencies should provide information and advice to people who are likely to be affected by flood.

P.3.4.002 (B)

Association of cognitive disorders (hypertension and depression) and marital status among elderly in India: do men suffer more?

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CONTEXT The rapidly unfolding phenomena of population ageing is associated with changing burden of non-communicable and life style diseases. Irrespective of increasing longevity, most of these extended years are spent in struggle with rising multi-morbidity conditions among elderly with substantial demographic, socioeconomic and contextual differences. Previous studies have noted differential positive effects of marital status on physical and cognitive health status of elderly. However, to what extent the change in marital status is associated with the risk of depression and hypertension among elderly men and women is not clear in Indian context.
OBJECTIVE The present paper examines the association of marital status with cognitive disorders (depression and hypertension) stratified by gender to highlight if elderly men suffer more than their female counterparts.
DATA AND METHOD Using the data from the World Health Organization sponsored study on conducted by IIPS Global Ageing and Adult Health (SAGE-India) covering 10 000 households in India, the study attempts to test the hypothesis of poor cognitive status (elevated risk of depression and hypertension) among widowed/separated/single elderly men as compared to elderly women in selected Indian states (Assam, Karnataka, Maharashtra, Rajasthan, Uttar Pradesh and West Bengal). We use bivariate analysis, chi-square tests and adjusted logistic regression models to validate our hypothesis.
RESULTS Substantial differentials in the risk of cognitive disorders by marital status of elderly men and women are found. On an average, the risk of cognitive disorders was higher among widowed/single/separated elderly men than their female counterparts. There is need for gender-specific policies to cater the cognitive needs of elderly population, particularly those who are widowed/separated and staying alone in the last phase of their life.

3.5. Hypertension, cardiovascular diseases and stroke

P.3.5.002 (B)

Hypertension and diabetes in Andean migrants: a model of chronic non-communicable diseases in a transnational context

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BACKGROUND Andean migrants in Europe may suffer chronic non-communicable diseases, thus needing different levels of health care. There is a paucity of data on this issue as well as specific health policies focused on the transnational context. The project 'coordinating resources to assess and improve health status of migrants of Latin America (COHEMI)' intends to respond to this issue assuming hypertension and diabetes as models for analysis.
METHODS Systematic reviews of epidemiological studies and clinical guidelines, as well as field studies and network interaction were considered to recommend health policies to the European governments.
RESULTS From systematic reviews, in Bolivia, Ecuador and Peru prevalence of hypertension was 1861 (95%CI:1290–24.32); and of diabetes was 3.52 (95%CI:3.52–3.53). Prevalence of hypertension in Latin American migrants in Spain was 6.12 (95%CI:6.12–6.13). No related literature was found in Italy; however data from pharmaceutical registries in 2011 showed that 4.96% of migrants regularly took drugs of the cardiovascular ATC-WHO group. Assessment of official clinical guidelines from Ecuador, Peru and the European Society of Hypertension using the AGREE tool showed a score of 3, 4 and 5, respectively being the implementation criteria the weakest domain. A community epidemiology based field study in Ecuador showed effectiveness for monitoring hypertensive patients.
CONCLUSIONS Hypertension and diabetes are important chronic non-communicable problems in Andean countries; however the burden of these problems in Latin American migrants in Europe may be lower. There is a need of community based indications for monitoring of such problems in the transnational context and the community epidemiology model from Ecuador may be useful. This work has been supported by the EC within the 7th Framework Program under the COHEMI project – grant agreement no. FP7-GA-261495.

3.6 The challenges of neurological diseases in resource-poor settings

P.3.6.001 (A)

Etiology and outcome of neurological disorders in the Rural Hospital of Mosango, Province of Bandundu, Democratic Republic of Congo: preliminary results

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INTRODUCTION Due to the lack of laboratory and imaging facilities, the etiological spectrum of neurological disorders is poorly known in rural Central Africa. To address this question, the consortium Neglected Infectious Diseases Diagnosis (NIDIAG) has set up a clinical and diagnostic study in the Democratic Republic of Congo (DRC) since September 2012.
METHODS All patients >5 years admitted with active neurological disorders are prospectively evaluated at the 'Hôpital Général de Référence' of Mosango, a 400-bed hospital located in the predominantly rural Province of Bandundu, DRC. All consenting patients are examined by a neurologist and submitted on site to a systematic laboratory workup on blood and cerebrospinal fluid (CSF). Patients are clinically followed-up until 6 months after discharge. Blood and CSF samples are sent to reference laboratories in Kinshasa and in Europe for further testing.

RESULTS Up to April 2013, 67 patients were enrolled [male: 54%; mean age: 38 years (range: 6–76)]. Mean presenting symptoms were altered consciousness (n = 28; 36%; including 11 with Glasgow Coma Scale ≤ 11), focal deficit (n = 14; 21%) and seizure (n = 11; 16%). Many patients (n = 34; 51%) had received antibiotics and/or antimalarials before admission. Infections were suspected or confirmed in at least 33 patients (49%), including mainly bacterial meningo-encephalitis [n = 13; 19%: Neisseria meningitidis (n = 7), Streptococcus pneumoniae (n = 1), presumptive (n = 5)], human African trypanosomiasis (n = 6; 9%), sepsis (n = 5; 7%), complicated Pott (n = 4; 6%) and tuberculous meningitis (n = 3; 4%). Other main diagnoses were epilepsy, cerebrovascular accident and neuropsychiatric condition (n = 7 for each; 10%). Eleven (16%) patients died, including six within 72 h after admission. Seven (10%) patients were discharged with severe neurological sequels.
CONCLUSIONS These very preliminary data show that treatable infections accounted for about half of the conditions causing neurological disorders in the Bandundu province, DRC. Outcome was often poor in this limited-resource setting with severely ill patients.

3.7 Cancer in a global perspective

P.3.7.001 (B)

A case–control study of cervical cancer and reproductive factors in women in Mozambique

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INTRODUCTION Cancer of the cervix is the most common cancer in women in sub-Saharan Africa. Reproductive history factors are often ignored in cervix cancer aetiology. The objective of this study was to identify reproductive factors in relation to risk for cancer cervix in adult women in Maputo, Mozambique.
MATERIAL AND METHODS In this study of cancer cervix, we investigated reproductive risk factors. Patients with cancer cervix (cases) and patients free of any cervical malignancy (controls) were recruited from the Central Hospital. The case-control study involved 133 cases and 120 age-matched controls. Data were collected by standardised interviews conducted by nurses.
RESULTS Between groups comparisons showed marked differences in reproductive history. The key findings were that risk of cervical cancer increased with low sexual debut age comparing age at first intercourse ≤15 years to ≥20 years (odds ratio 4.8; 95% confidence interval 2.1–11.3), with number of births ≥5 compared with 0–1 birth (odds ratio 4.0; 95% confidence interval 2.0–5.4). Women reporting having six or more sexual partners had a nearly six-fold increased risk of cervix cancer as compared with women reporting 1–5 sexual partners (odds ratio 5.7; 95% confidence interval 2.1–15.6). The association between cervix cancer and multiple sex-partners is likely explained by the elevated risk of infections, and early age at first coitus may strengthen this association as the immature cervical epithelium represents an increased susceptibility and vulnerability to both sexually transmitted deseases and oncogenic agents. Mean age of first delivery varied, the cases had their first child at age 18.8 and controls at age 20.8.
CONCLUSION Reproductive histories varied between cases and controls. The cases had more children, deliveries at a younger age, earlier sexual debut and more sexual partners than the controls. In short: the cases had more often a high risk reproductive profile than the controls.

P.3.7.002 (B)

Endemic Kaposi's sarcoma in Central Africa Republic: unusual manifestations and response to Bleomycin

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BACKGROUND Kaposi's sarcoma (KS) is an angioproliferative disease. Four clinical types are recognized: classic (sporadic, mainly in elderly patients), African (endemic, mainly in Sub-Saharan Africa), iatrogenic (immunesuppression-associated), and epidemic (AIDS-associated). We describe a case of unusual mucous-cutaneous KS dissemination in an HIV-negative patient from Central African Republic (CAR), treated with bleomycin.
PATIENT AND METHODS A 21 years old African patient, from Bozum (western CAR) presented with blackish-purple nodular skin lesions spread into the whole body and similar mucosal lesions of the oral cavity. He also had a bleeding proliferative lesion in the right lower eyelid conjunctiva, all suggestive of KS. In November 2012 he was subjected to serologic (DETERMINE and UNIGOLD rapid test), virologic (HIV-RNA with VERSANT k-PCR) and immunologic screening for HIV infection (Facscuont Bekton Dikinson). HHV-8 was searched by real-time PCR.
RESULTS The patient was HIV negative with CD4+ >1000/mmc. HHV8 DNA was detected in plasma (1.380 copies/ml). Histological analysis of two flaps of skin confirmed Kaposi's sarcoma. From November 12 to February 13 the patient was subjected to six cycles of bleomycin (15 mg), performed every 15 days, upon control by complete blood count (CBC), aminotransferases (AST, ALT) and creatinine. A sensible improvement of skin and membrane lesions was obtained.
CONCLUSIONS We describe diagnosis and treatment of an endemic-KS in a HIV-1 negative subject with an uncommon muco-cutaneous diffusion, that showed a good response and tolerability to the treatment with bleomycin.

P.3.7.003 (B)

Quality of life of prostate cancer patients in Shanghai, China

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BACKGROUND With the population aging and economic development, it is very likely that the disease burden of prostate cancer will keep rising in China. This study aims to assess the quality of life of prostate cancer patients in Shanghai.
METHODS A cross-sectional study was carried out in two districts of Shanghai. Face-to-face interviews were given by trained investigators to the consented participants. Quality of Life was assessed by Functional Assessment of Cancer Therapy C General (FACT-G) and Prostate Cancer-Specific (PCS).
RESULTS Of the 440 prostate cancer patients registered during 2007–2009, 122 (27.7%) died before the investigation. In total, 222 prostate cancer patients consented to participate in the interview, and the mean of age of the study subjects was 73.85 years old at diagnosis and 76.50 at interview. Of them, 79%

(162) initiated their healthcare seeking due to symptoms, the other 21% (60) were suspected for prostate diseases by routine health checkup. The mean scores for general physical well-being (GP), social/family well-being (GS), emotional well-being (GE) and functional well-being (GF) were 22.49, 18.71, 19.47 and 17.43 respectively. The subtotal of FACT-G in mean was 78.13. The mean score for PCS was 34.41. The total score of FACT-G and PCS was 112.38. Significant correlations were observed between severity of cancer and quality of life. The higher the stage was, the lower the FACT-G score would be. Metastasis and relapsed was associated with lower FACT-G score. The similar correlations were found in the assessment using PCS.
Conclusion The quality of life of recent diagnosed prostate cancer patients was relatively low. With the increasing incidence of prostate cancer in Shanghai, and the rapid population aging, it is imperative to develop evidence-based policy and strategy to improve the quality of life of prostate cancer patients.
Key words Prostate Cancer, Quality of Life, FACT, China.

Track 4: Health systems and resources

4.1 Health systems and integrated control of diseases

4.1.2 Blood transfusion

P.4.1.2.001 (B)

Knowledge, attitudes and practices about blood donation among the Ndjamena population in Chad

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Introduction In Chad, like in every country of Sub-Saharan Africa, the need for blood is enormous. The voluntary and unpaid blood donation which is one of the ways to access to safe blood transfusion represents <5% of the collected blood. This study aimed to analyze the knowledge, attitudes and practices of N'Djamena population regarding voluntary blood donation and to identify obstacles to this procedure.
Methods This cross-sectional and descriptive study was conducted from May 2nd to July 13th, 2012 in N'Djamena. It was done on a sample of 400 schooled students and persons aging between 17 and 60 years frequenting the National Center of Blood transfusion.
Results Three hundred and forty persons aged from 17 to 60 years (264 males, 76 females) filled the questionnaire. Of 75% were under 25 years old, 91% agreed with blood transfusion and 79% were willing to donate blood. 132 (39%) persons were already donors of which only 18% a voluntary and unpaid blood donation. The reasons for donation refusal were related to the fears of blood (30%), needles (24%) and viral transmission (21%) as well as the lack of information.
Conclusion This study shows that several barriers prevent the practice of voluntary blood donation. New strategies are needed in order to inform and motivate the donors.

4.1.3 Access and use of health services

P.4.1.3.001 (A)

Improving clinical governance of HIV treatment programs in resource poor settings: the role of digitising clinical notes

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Ensuring good standards of clinical governance is crucial to providing effective health care in resource poor settings. Unfortunately, implementing clinical audit in these settings is often beset by practical issues. We propose a method of out-sourcing the burden of data analysis to facilitating regular audit in resource poor settings. Critical to this is the development of a cheap and quick system to gather data for remote auditing. We investigated the feasibility of using an inexpensive 12-megapixel point-and-shoot digital camera to collect data from clinical notes in a format capable of being sent via secure electronic file transfer for remote analysis. We photographed a random selection of 20 anonymised clinical records, from among 1000, of the HIV outpatient clinic of Cecilia Makiwane Hospital, East London, South Africa. We recorded the time taken to retrieve and photograph each record and evaluated digital images for legibility and whether information on use of the antiretroviral drug Tenofovir, suitable for clinical audit, could be retrieved from these images at a later time. Specifically, we audited whether antiretroviral drug name and creatinine clearance were recorded at baseline, months 3 and 6, and then every 12 months thereafter, in line with the latest South African Department of Health Standard Treatment Guidelines. The time to retrieve and photograph each of the 12 records ranged from 90 s to 5 min, with a mean of 2.5 min. Seven of the patients were receiving tenofovir. None of the records indicated, either in the notes or by inference from results, that blood for creatinine clearance measurement had been taken according to clinical guidelines. In this proof of concept feasibility study we demonstrated a reliable, cheap and quick method to digitise clinical records in an electronically transferable format, offering a way to facilitate standardised and large scale collection of auditable data.

P.4.1.3.002 (B)

What happens when high coverage of health facility delivery is achieved? Preliminary findings from a community and hospital survey to assess equity of comprehensive emergency obstetric care in rural Tanzania

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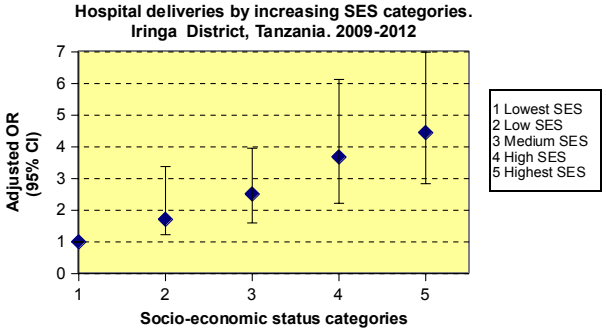
Introduction As MDG5-related strategies take effect, coverage of maternal services will increase among the rural poor. However, it is not known whether in high coverage all socio-economic groups will access services equally. Iringa District, Southern Tanzania, with a rural population of 260 000 and high health facility delivery coverage, is a paradigmatic area to address this question. In 2009 a District representative household survey documented 88% of facility deliveries. Delivery services are available in 65 primary (dispensaries, health centres) and one secondary (hospital) facility.
Methods Socio-demographic characteristics of the District population were obtained from the household survey (Community survey, $n = 463$). Data on women who had

Table 1 Characteristics of women who delivered at the District hospital compared to women from the community of origin. Iringa District, Tanzania 2009–2012

Characteristics of study participants	Community survey (N = 463) n (%)	Hospital survey (N = 1072) n (%)	OR adjusted* (95% CI)	P-value†
Education (years)				
0	58 (12.5)	74 (7.0)	0.88 (0.61–1.25)	0.4584
1–6	35 (7.6)	22 (2.1)	0.33 (0.23–0.49)	0.0000
7	353 (76.2)	821 (78.0)	1 (–)	–
≥8	17 (3.7)	135 (12.8)	1.93 (0.97–3.86)	0.0616
Sex of household head				
Female	49 (10.6)	77 (7.4)	1 (–)	–
Male	412 (89.4)	965 (92.6)	1.74 (1.01–3.00)	0.0468
Type of delivery				
Vaginal	420 (90.7)	736 (68.7)	1 (–)	–
Cesarean section	43 (9.3)	335 (31.3)	1.96 (1.62–2.37)	0.0000
SES				
Lowest	91 (19.8)	64 (6.1)	1 (–)	–
Low	92 (20.0)	120 (11.5)	1.71 (1.23–2.38)	0.0022
Medium	84 (18.3)	180 (17.2)	2.51 (1.60–3.96)	0.0003
High	88 (19.2)	252 (24.1)	3.68 (2.22–6.13)	0.0000
Highest	104 (22.7)	428 (41.0)	4.45 (2.84–6.99)	0.0000

*Adjusted for age, parity, education, sex of household head, type of delivery and SES.
†Adjusted Wald test.



delivered at the only Comprehensive Emergency Obstetric Care facility was collected using a questionnaire between Oct 2011 and May 2012 (Hospital survey, n = 1072). Characteristics of women from the two surveys were compared. Socio-economic status (SES) was assessed using principal component analysis on household assets. The community sample was divided into five quintiles. Community quintile cut-off values were applied to the hospital sample. Multivariable logistic regression was performed to examine differences between the two groups.

RESULTS Socio-demographic characteristics were significantly different between the two groups. In the Hospital survey compared to the community sample, women were significantly younger, had lower median parity, and were more likely to belong to higher SES categories. The adjusted odds ratio (OR) of delivering at the hospital increased progressively across the SES categories, from 1.71 for the low SES (P = 0.0022) to 4.54 (P < 0.0001) for the highest SES (Table 1).

CONCLUSIONS Where facility delivery coverage is high, there is significant inequity in comprehensive emergency obstetric care services' access. Poorest women are significantly under-represented where life-saving blood transfusions and caesarean sections are available. Strengthening of care in Dispensaries and Health Centres, including emergency transport, is paramount to reduce the equity gap.

P.4.1.3.003 (B)

Eliminating user fees for facility-based delivery services: a difference-in-differences evaluation from five West African countries

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INTRODUCTION Several countries in sub-Saharan Africa have recently adopted policies that remove or substantially reduce user fees for facility-based delivery services. There is little rigorous evidence of the impact of delivery fee exemption policies on utilization of facility-based delivery services and no previous evaluations have examined effects on neonatal mortality rates (NMR). The objective of this study is to estimate the causal effects of a delivery fee exemption policy on the proportion of births delivered in a health facility, the proportion of deliveries by Caesarean section, and NMR.

MATERIALS AND METHODS We used data from Demographic and Health Surveys conducted in five West African countries between 1998 and 2011. Burkina Faso, Ghana, and Senegal adopted policies removing or substantially reducing user fees for facility-based deliveries and Caesarean sections between 2003 and 2007, while Nigeria and Cameroon did not adopt user fee policies and were used as controls in the analysis. We used a difference-in-differences (DD) regression approach to control for underlying secular trends in the outcomes that are common across countries and for time invariant differences among countries.

RESULTS According to covariate-adjusted DD models, the policy change was associated with increases of 7.5 health facility deliveries per 100 live births [95% confidence interval (CI) 4.8, 10.1] and 0.5 Caesarean deliveries per 100 births (95% CI 0.1, 0.9), and an estimated reduction of 3.8 neonatal deaths per 1000 live births (95% CI −8.2, 0.5). We performed several checks of the DD model assumptions, which generally supported our main results.

Conclusions Our findings suggest reducing fees for delivery services led to substantial increases in facility-based deliveries and Caesarean sections, and possibly contributed to a reduction in neonatal mortality. Evidence from this and other user fee policy evaluations will be useful to governments weighing the potential benefits of abolishing user fees for delivery services.

P.4.1.3.004 (B)

Impact of model household training by health extension workers on immunization coverage, nutritional status, and insecticide treated-nets utilization in Dera District – Ethiopia

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Background Having been implemented as the element of the Health Extension Program since 2003, the impact of model households training has not been evaluated. This study was objected to evaluate the impact of the model households training on immunization coverage, nutritional status, and Insecticide-Treated Nets (ITNs) utilization.
Methods We did a quasi-experimental study comparing three health-related outcomes in 60 model households with 60 non model households in Dera District, Ethiopia.
Results and Conclusion The model households training showed positive impact to the full immunization coverage. The difference between proportions of fully immunized children in the model households group and in the non model households group was significantly different ($P < 0.05$). The children in the model households group were more likely to be fully immunized compared to the children in non model households group (OR 2.85, 95% CI 1.028–7.904). From four variables analyzed; status of being model households, literacy, wealth, and age, only the status of being model households was significantly correlated with the full immunization coverage ($P < 0.05$). The impacts of model households training to nutritional status and ITNs utilization were not detected ($P > 0.05$).

P.4.1.3.005 (B)

Utilization of curative care and health service entry point preferences in Centro Habana, Cuba

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In the Cuban national health system the family doctor is seen as essential entry-point. Alternative entry points are the policlinic and hospital emergency services. We analysed health seeking behaviour in function of perceived health problems. In Dragones, Centro Habana, a transversal study was set-up using a systematic sample of 408 families (1244 persons). During home interviews between April and June 2010 information was collected on socio-demographic data, on health problems over the last month and on health seeking behavior. Five hundred and twenty-nine persons reported a chronic disease (mainly hypertension, asthma, diabetes or arthritis), of which 155 had an exacerbation of their chronic problem over the last month and 50 had an acute health problem. Also 107 persons without chronic disease reported an acute health problem over the last month. Elderly and women had significantly more chronic problems, and higher educated persons had less. Persons with higher education and singles had significantly more chronic exacerbations, while adult women and elderly had more acute problems. Two hundred and thirteen patients consulted formal health services [111 (70.7%) and 102 (65.8%) for acute and chronic problems respectively]. Acute patients with an underlying chronic disease used more formal services [OR 0.39 (0.17–0.92)]. Family doctors were the entry point for 65 (58.6% of formal contacts) acute and 50 (49.0%) chronic patients. Alternatives were the policlinic [16 (14.4%) acute and 20 (19.6%) chronic cases] and the hospital [30 (27.0%) acute and 32 (31.4%) chronic cases] emergency services. Males were more likely to visit a policlinic or hospital [OR 2.3 (1.099–4.815)]. The entry-point function of the Cuban family doctor needs to be strengthened. The organizational set-up - with technical support concentrated at policlinic level - and existing problems with long term stability of family doctors in their community are identified as essential issues.

4.2 Health financing

P.4.2.001 (A)

Towards universal health coverage in low income settings

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Introduction The Rwanda's long term Vision 2020 in health sector is to provide sustainable financial resources for universal access to health care. The goal of Rwanda Community-Based Health Insurance (CBHI) Policy is to provide a national framework for strategies and actions aimed at assuring that all residents of Rwanda can be enrolled in a health insurance scheme that provide quality health care.
Objectives To document the Rwandan experience in achieving its long term objective to attain the universal access to quality health care.
Material and Methods The study is descriptive, documentary and retrospective (2000–2012).
Results (i) Policies, laws, strategies and actions have been elaborated and implemented in order for each Rwandan citizen to access quality healthcare and to be protected from financial risks due to illness. (ii) Average enrolment rate to CBHI raised from 7% (2003) to 91% (2012) and CBHI members are benefiting adequate package of healthcare provided by public and Faith-Based Health Facilities countrywide, from primary to tertiary levels. (iii) Vulnerable, estimated at 24.8% of Rwandan population, are all enrolled in CBHI in year 2011/2012.
Core Factors Strong government leadership, political commitment, good governance/stewardship, consistent Government's financial subsidies and international aid aligned to priorities with a regulatory framework, their efficient and transparent coordination and synergy between reforms in health sector.
Looking Ahead However, there remains some challenges: (i) Increasing and diversifying internal financial resources, (ii) strongly adhering to international aid commitment, (iii) maintaining current package and quality health care, (iv) monitoring and measuring effects of recent CBHI's premiums based on population's stratification.
Conclusions The above-mentioned core factors and challenges should serve as lessons, not only to Rwanda, but also

to any country in comparable settings embarking in the universal health coverage.
Key words Health Insurance, Rwanda, Universal Health Coverage

P.4.2.002 (A)

Introducing Islamic governance to administer and manage the collection of Zakat (mandatory Muslim tax) in order to finance reproductive health care in Kenya

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Rights cost money and in achieving the right to reproductive health care the Kenyan government has realised that finance plays a critical role. Thus, it is in this context that this paper will introduce zakat as a method of financing the right to reproductive health care in Kenya. Since zakat is an Islamic form of mandatory taxation its administration and management by the Kenyan government must be done in accordance with the principles of Islamic governance in order to encourage Muslims to pay their zakat to the secular state. Accordingly, this paper will explain how to restructure Kenya's National Health Insurance Fund with principles of Islamic governance to finance reproductive health care using zakat. Hence this paper presents an idea for future research based on expanding the tax base of countries by introducing zakat into their health policies and to further develop the concept of a hybrid system of health governance containing both secular and Islamic principles. This study shall utilise a mixed method approach, with an emphasis upon qualitative methods based upon archive research, which shall include health and governance legislation, health regulation, ministerial circulars on health and governance, newspapers, official and research documents both local and international related to health and governance and semi-structured expert interviews with policy makers, relevant government ministries, tax experts and officials. The quantitative methods shall involve analysing the total amount collected as zakat in Kenya. The results may prove or disprove the research hypothesis that zakat is not just a faith based tax but is potentially a legal, reasonable and rational tax which although different from conventional taxation may provide the financing required for reproductive health care in Kenya.

P.4.2.003 (A)

Universal health coverage in Rwanda: dream or reality

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Introduction Universal Health Coverage (UHC) has been a global concern for a long time and even more nowadays. The Rwanda commitment to move towards UHC appears through various long term strategies, policies, laws and arrangements focusing on a strategic social protection through universal access to healthcare from 2000 to present. While a number of publications are almost unanimous that Rwanda is not far from UHC, very few have focused on its financial sustainability.
Objectives (i) To make a critical scrutiny of Rwanda UHC based mainly on Community-Based Health Insurance (CBHI) from 2000 to 2012 by highlighting its progress, strengths, weaknesses, opportunities and threats (SWOT); (ii) to inform policy makers about observed gaps for a better way forward.
Methods A retrospective (2000-2012) SWOT analysis was applied to six metrics as key indicators of UHC achievement related to WHO definition of UHC, i.e. (i) access to care or insurance, (ii) equity and/or risk equalization, (iii) package of services, (iv) rights-based approach, (v) quality of health care, (vi) financial-risk protection, and a seventh metric related to Rwanda CBHI self-financing capacity (SFC) was added by authors.
Results The first metric with 90.75% of CBHI rate and 1.07 visits per capita per year versus 1 visit recommended by WHO, the second with 24.8% indigent people subsidized versus 24.1% living in extreme poverty, the third, the fourth, and the fifth metrics excellently performing, the sixth registering a very good performance with only 10.80% versus ≤40% as limit acceptable of catastrophic health spending level and lastly the CBHI SFC i.e proper total cost recovery, estimated at 82.55% in 2011/2012, Rwanda UHC achievements are objectively convincing.
Conclusions Rwanda UHC is not a dream but a reality if we consider all convincing results issued of the seven metrics.
Keywords Universal Health Coverage, Community-Based Health Insurance, Rwanda.

P.4.2.004 (A)

Health insurance in India: opportunities and challenges

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Background At present, Indian health system is burdened with communicable and non communicable diseases; the supply of health facilities falling short of demand. This has forced people to pay much more than their capacity. Health insurance, in such situations, comes out to be the safest option for health care financing. One of the important challenges is to convert predominantly private out of pocket (OOP) spending into health insurance premium.
Objective (i) To assess the importance of financial issues in purchasing health insurance (ii) to understand insurance cover of 45+ populations (iii) to explore the factors that restrict the growth of insurance sector.
Data and Methods The study has used pilot data of Longitudinal Ageing study in India, 2010 conducted by International Institute for Population Sciences, Harvard School of Public Health and RAND Corporation focused on social, economic and health experiences of 45+ populations. The study has employed univariate and bivaraite techniques on 1585 respondents as per the data availability.
Results The study reveals that 61% of the respondents consider financial issues, an important factor in purchasing insurance cover. Only five percent of study population has insurance which means, by and large, irrespective of socioeconomic standing, people are not really accepting the concept of health insurance. The most important reason for low level of acceptance is lack of knowledge (65%) followed by affordability and accessibility.
Conclusion Health insurance in India is in its embryonic stage. Majority of the study population is not aware about health insurance and lack of awareness often leads to lack of demand. There is huge potential of developing the health insurance market. India has one of the highest domestic savings rate; the need of the hour is to channelize these savings into a proper direction where people can learn to invest for their own health.

P.4.2.005 (A)

Cameroonian communal health insurances and managerial performance, a clashing couple

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Background Communal health insurances (CHI) were created in Cameroon to improve the health condition of the most impoverished persons. In 2001, the government has set goals for 2015 as the establishment of a community health insurance in each of the 178 district health departments in Cameroon, and the coverage of at least 40% of the population. It therefore becomes necessary to regularly monitor the performance of CHI to achieve these objectives.
Methods The aim of this study was to make an assessment of managerial performance of CHI based on four indicators: number of members, penetration rate, claim rate and expense ratio. Six CHI representing the geographic, linguistic and cultural diversity of Cameroon were selected: Edea, Kumbo, Loum, Manjo, Mbanga and Ngaoundéré.
Results At the end of this study it appeared that the number of members and the penetration rate were respectively <1000 and 1%, except Kumbo with 11 280 members and penetration rate of 7.52%. All CHI claim rates were higher than the 75% standard except Ngaoundéré, which demonstrated the difficulty for managers to control the information asymmetries. Finally, all CHI had difficulty to maintain the expense ratio below 15%.
Conclusion The performance level of Cameroonian CHI are insufficient. These health insurance companies are at risk of bankruptcy. Recruitment of new members and reinforcement of managerial skills are necessary.

4.4 Translating what we know into global action. Implementing research into policy and practice - roles of media, researchers, decision-makers and civil society

P.4.4.001 (A)

Integrating health in impact assessments, opportunity for health promotion post 2015

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Brief Introduction Prospective Impact Assessment (IA), i.e. the systematic application of foresight to human activities on societal level, is worldwide used and agreed upon as necessary and useful approach. Health is the focus in Health Impact Assessment (HIA) but also touched upon in most IAs. Health inclusion in IAs is challenging, given its broad, cross-cutting nature. Since health can be 'produced' as well as 'damaged' or even 'destroyed' by multiple societal sectors, health inclusion in IAs is essential. This is especially important for the sustainable development agenda post 2015 in those countries where only the mainstreamed IAs are conducted.
Material and Methods Internationally recognised IA specialists were asked to analyse how health is addressed in their respective fields. The analysis was structured around key questions identical for each IA type to allow further comparison. These were presented by the researchers and discussed with other IA practitioners. The joint discussion further refined the papers, the overall conclusions, and recommendations on the way forward.
Results The research showed that health considerations are included in various IAs in many different ways, and neither a unique definition of health nor a unified approach exist. There is comprehensive research, and practical experiences, many of which not well-known beyond 'inner circles'. Several questions remain unanswered. Most importantly, there is no single univocal answer to the question how best to bring together health and IAs.
Conclusions Better inclusion of health issues in IAs will support the sustainable development agenda post 2015. The following elements need to be further clarified:
1 explicit discussion of human health impacts;
2 comprehensive consideration of health determinants;
3 causal pathways from health determinants to health outcomes, including interactions;
4 distribution of health impacts on various groups (health equity);
5 utilization of health data to inform the analysis and possibly quantify health impacts.

P.4.4.002 (A)

Reporting of health care research advancements in newspapers, perception and practice of indian housewives – a qualitative study

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Introduction Newspaper and magazine reports about advancements in health care and medical researches are more exposed to public. The association of practice followed by public, their perception about news on new findings and how the media reporting these have to be studied intensively.
Materials and Methods The study was divided in to three phases. First phase contains qualitative content analysis of media reports of health related research advancements published on three most circulated national newspapers from national capital region (New Delhi) of India for 3 months. The second phase was in-depth telephonic interviews conducted on 60 housewives aged above 40 years for understanding perception of media reports. The third phase was expert validation and quantitative scoring of the in-depth interviews based on pre-prepared scales. This study make use of methodological triangulation for validation of association between media reporting, perception and practice.
Results and Conclusions The media's preference in reporting research advancements are associated with the practice of Indian housewives. The result reveals that, their belief about research outputs are highly biased towards their judgments based on self-evaluation without any medical knowledge. The associations of disease prevalence and household substance uses are frequently reported by media, such nominal associations found to be affecting modification in diet preference by 60% of housewives.

P.4.4.004 (A)

The context assessment for community health tool – investigating why what works where

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Background Systematic reviews have found that common strategies used for knowledge translation (KT) in healthcare (i.e. training, reminder systems, feedback of results) generate a median effect of 4–6% improvement. The gap between what is known and what is practiced results in patients not benefitting from advances in healthcare and being exposed to unnecessary risks. Furthermore, healthcare systems bear unnecessary expenditure. The Context Assessment for Community Health (COACH) tool was developed on the premise that organizational context influences KT. Prior to the COACH tool there were no such tool available for low-income settings. Tools to measure contextual factors influencing KT have however been developed, and are being used, in in high-income settings.
Methods The COACH project was initiated by the Research for Improved Child network. The core research group members consisted of researchers from Bangladesh, Vietnam, Uganda, South Africa, Nicaragua, Canada and Sweden. The group developed and initially validated a tool to assess factors in the local health system that influences KT. We have undertaken content validity assessments amongst expert panels in each of the settings and international experts in implementation science. We are currently undertaking psychometric evaluation of the tool based on data from 150 health workers in each of the five settings.
Results The COACH tool measures the following nine aspects of context: leadership, work culture, monitoring services for action, interaction between members of the unit, sources of information, resources, community engagement, commitment to work and informal payment.
Conclusion We foresee that the COACH tool can be applied to; (i) address and act on locally identified shortcomings of the health system to increase effectiveness for health system strengthening, (ii) guide planning and promote adaptation of interventions to the local context in low-income settings and (iii) evaluate, understand and compare process and outcome indicators of healthcare interventions in low-income settings.

4.5 Health systems in conflict and disaster areas

4.5.1 Health systems in conflicts and natural disasters

P.4.5.1.001 (B)

Repeated outbreaks of infectious diseases reveal need to create improved response capacity of the health system in Democratic Republic of Congo

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Introduction As in other post-conflict contexts, precarity and health needs of the population are high in DRC. This is shown by regular emergence of epidemic outbreaks, for which MSF support is often requested. Health systems strengthening is high on the international agenda and also the main strategy for the health sector in DRC, chosen by government and development partners. How to support weak health systems is a particular challenge in so-called fragile states.
Material and Methods During outbreaks, MSF intervention teams, take stock systematically of the health structure in terms of availability of basic drugs and other medical commodities, financial accessibility and support/supervision provided. We did a review of all health structure reports collected in 2011 and 2012. Additionally the circumstances of the alert and the reactive response of the health system to the outbreak were studied. Comparisons were made in reactiveness of the health structure depending on the international support received.
Results and Conclusions The analysis shows a lack of availability of essential drugs, such as anti-malarials, painkillers and antibiotics, with regular stock outs. Available medicines are difficult to access because of high patient fees. As a result of low utilization rates, the surveillance system based on health structure data fails to pick up increases in infectious diseases. When alert is given, there are important delays in response from the health system; additional supplies are late to arrive, there is reluctance to waive fees to allow cases to come and get treated and mobilization of additional resources from international partners already active in the district proved slow. The presence of international donor support did not show a significant difference in response potential. A critical review of health systems strengthening efforts is needed, in order to include measures and mechanisms to improve effective reaction to outbreaks of the health services.

P.4.5.1.002 (B)

Middle-level practitioners' roles for sustainable human resources for health in rural settings: Okinawa at postwar and Ghana at present

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The history of health and health care in Okinawa from the end of World War II to the present offers many lessons that could be useful in planning, managing and providing health care in developing countries. Many of the problems faced then by Okinawa are still major problems in many countries those problems included scarcity and mal-distribution of health personnel, and generally poor public health. Just after the war, there were only 64 doctors in Okinawa. This was a third the number before the war. As the few doctors available shifted to work in the hospitals that were being built or rebuilt, doctors who could work at the clinics in remote islands and other remote areas were in increasingly short supply. As an immediate measure to deal with that problem, public health nurses (PHNs) have played key roles in disease prevention and control and in providing health services in remote areas of Okinawa, by managing their postings and provide the professional supervision and support they needed. Several lesson learned from the Okinawa's PHN posting system; leadership, quality and dedication of trainees, clearly defined role and responsibilities, safety, adequate housing and workplaces in communities, regular contacts and exchanges with supervisors and colleagues, etc. Although this 'excellent' system was abolished in Japan when the health systems were decentralized by enforcement of public health law in 1997, the similar effective approaches to tackle with the difficulties in posting

'qualified' mid-level practitioners to communities have been existing, such as in Ghana; in order to functioning community-based health planning and services (CHIPS) with posting community health officers (CHOs) at remote areas. The presentation will be focused on the comparison among some challenges of posting human resources for health in remote settings, and what are the key issues to achieve the goals through Okinawa's lessons.

P.4.5.1.003 (B)

Trusting in times of conflicts: the case of a long term conflict in the public health care sector in rural Burkina Faso

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Since 2011 the public health system in Burkina has been marked by on-going conflicts with strikes and full closure of all public health facilities orchestrated by the unions of the health care providers and in one case an attempt to burn down a dispensary by angry community members. How has the conflict(s) in a country already affected by regional instability and widespread poverty marked the ways people perceive state sponsored services? This study seeks to complement existing knowledge on utilization of primary health facilities in developing countries with an investigation of whether the concept of trust is of relevance to the study of public health services, especially in times of conflicts. The study is based on an on-going doctoral work in a rural area using ethnographic methods including long term fieldwork, in-depth individual interviews with patients and therapy managers, FGDs, document analysis and clinical observations combined with a survey. The study found that in spite of the fact that the public dispensary has little to dispense people increasingly seek treatment here, especially for signs of fever and for infant sickness. The patient-provider interaction is characterized by asymmetric power and patients are routinely subject to rough treatment. Because of the unequal power relations people have to trust the good intentions of the providers. However, the longer the conflict lasted the more people started to doubt their good intentions and loyalty to the community. Rather than representing a radical rupture in the way people expect the health system to function, the conflict was perceived as a continuation of the disappointments that people in rural areas encounter in their contact with public sector. The conflict was found to have a negative impact on the way people in rural perceive state sponsored health services and trust between users and the system was gradually broken.

4.6 Health and the environment

P.4.6.001 (B)

Improve health by acting on the environment: a cooperation project in a rural area of Burkina Faso

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Diarrhoeal diseases are responsible for the death of more than 2 million people each year. about 90% is related to unsafe drinking-water supply and sanitation. In Burkina Faso, a safe drinking-water source is not available for 39% of people and only 17% have access to sanitation. The project 'Water, health, hygiene and socio-economic development in the rural area of Béguédo', developed by Medicus Mundi Italia, 'Tovini' Foundation, SIPEC Foundation, Dakupa NGOs and Association of Burkinabè in Brescia, with the University of Brescia, aims at improving health and hygiene conditions and the access to safe drinking-water.

MATERIAL AND METHODS Microbiological analyses were carried out on the sources used by the people and along the supply chain. Questionnaires were administered to assess the knowledge, attitude, practice in the management and use of drinking-water, sanitation, hygiene and health: 200 interviews at population (1 household for yard), 9 at school teachers, 36 at students (four kids for classes).

RESULTS Faecal contamination increased from on average 2 *E. coli* CFU/100 ml at sources, to 50 in transportation tanks and 200 in storage tanks. Significant statistical correlations were found between 'gastroenteritis' and the variables: use of shared latrines ($P = 0.046$); cup for drinking left on the ground ($P = 0.036$); cup for drinking deposited on the storage tank with the side facing upwards ($P = 0.016$); presence of animals close to the storage vessel ($P = 0.001$).

CONCLUSION The absence of significant statistical correlations between 'gastroenteritis' and the type of source indicates that the source cannot be regarded as the unique cause of diarrhoeal diseases, indeed there are external factors that contribute to modify the quality of water. Several awareness trainings were organized, based on the results obtained from the interviews and the analyses of drinking water, and addressed to all the stakeholders; a follow-up survey will be performed in May–July 2013.

4.7 Unexpected observations in global health: A call to report and react

P.4.7.001 (A)

The effect of Diphtheria-Tetanus-Pertussis (DTP) booster dose at 18 months of age on adaptive pertussis immunity in Guinea-Bissau

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Resurgence of pertussis has been described in several countries despite good coverage of the primary series of DTP (diphtheria, tetanus and pertussis vaccine) (DTP1-3). Currently WHO recommends a booster dose of DTP between 1 and 6 years of age after completion of DTP1-3. From 2006 DTP booster was no longer provided in Guinea-Bissau. We aimed to examine the effect of providing a DTP booster at 18 months of age on antibody levels against B. pertussis 6 months after vaccination. Eighteen-months-old children registered by Bandim Health Project's demographic surveillance system in Bissau who had completed DTP1-3 were randomised to receive either DTP booster+OPV (oral polio vaccine) or OPV only. Blood samples were collected from 508 children and analysed for levels of IgG antibodies to pertussis toxin (anti-PT IgG) before randomisation and 6 months

later. Anti-PT IgG levels above 10 IU/ml were used as an indication of protection. By 18 months of age, only 30% had an anti-PT IgG level above 10 IU/ml. Six months later, 57% of children who had received DTP booster had anti-PT IgG level above 10 IU/ml compared with 23% among children who had not received DTP booster, the relative risk (RR) being 2.43 (95% CI = 1.90–3.12). Antibody levels did not differ by sex. Underweight children (*Z*-score below −2) had significantly lower initial levels of antibodies than normal weight children, but the antibody response to vaccination was the same in underweight and normal weight children. These results show that many children have antibody levels which indicate that they could be susceptible to pertussis. As vaccines may have both specific and non-specific effects, an evaluation of the need for a booster vaccine needs to assess the effect of this vaccine on overall mortality and morbidity.

P.4.7.002 (A)

Will GAVI support for higher DTP-3 coverage lead to lower coverage for measles vaccine and higher child mortality? An observational study of the introduction of new vaccines in Guinea-Bissau

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Introduction The main performance indicator for the vaccination programme is the coverage of the third dose of diphtheria-tetanus-pertussis vaccine (DTP-3) at 12 months of age and not the coverage of measles vaccine (MV). Children in Guinea-Bissau no longer receive routine vaccines after 12 months. We have questioned this policy because many studies have shown that MV lowers child mortality, whereas no prospective study has found that DTP reduces mortality. In 2008 GAVI funded improved outreach services and introduction of pentavalent (DTP-HepB-HiB) and yellow fever vaccines in Guinea-Bissau.

Methods Bandim Health Project maintains a surveillance system with assessment of vaccination status and mortality in 182 randomly selected village clusters in rural Guinea-Bissau. We assessed vaccination coverage by 12 months of age among 878 children born 2007 and among 879 children born 2009. Among 12 386 children followed after 12 months of age in 1999–2006 we assessed MV coverage between 12 and 36 months of age and compared mortality of measles-vaccinated and measles-unvaccinated children.

Results Fifty-three per cent (468/878) of children were fully vaccinated prior to the introduction of the new vaccines and 53% (467/879) after. The timeliness of DTP improved and DTP-3/pentavalent-3 coverage increased from 73% in 2007 to 81% in 2009 ($P = 0.001$). However, the age of measles vaccination increased and coverage declined from 71% to 66% ($P = 0.10$). Hence the effect differed for DTP-3 and MV ($P = 0.002$). In the 1999–2006 cohort most children who were measles-unvaccinated by 12 months of age, received MV within the next year. Mortality was significantly lower for measles-vaccinated than measles-unvaccinated children, the mortality rate ratio (MRR) being 0.74 (0.59–0.92) overall, 0.62 (0.45–0.87) for girls and 0.91 (0.66–1.25) for boys.

Conclusion The coverage and timeliness of DTP vaccinations increased but the median age of MV increased and the MV coverage dropped. This may increase mortality.

4.8. Global and International Health

4.8.3 Universal Health Coverage: yes, but coverage of what? The need for People-Centered Care

P.4.8.3.001 (B)

Universal health coverage in a high-income nation: perspectives of people of refugee and ethnic minority backgrounds living in urban Australia

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Introduction Refugees and minorities living in high income nations that purport to have universal health coverage (UHC) frequently experience barriers to accessing healthcare and quality health services. Marginalization from the health system can contribute to diminished health status, which detrimentally impacts on the ability of minority populations to sustain employment, educational opportunities, and be included in ordinary community life.

Material and Methods This presentation will discuss findings from an Australian-based qualitative research study that forms part of Work Package 2 of the Go4Health Project (Go4Health: 'Formulating new Goals for Global Health, and proposing new Governance for Global Health that will allow the achievement of these goals'). WP2 aims at consulting marginalized communities, feeding lay perspectives into Go4Health recommendations. Consultations are being carried out in Africa, Latin America, Asia, and the Pacific. The University of Queensland is leading consultations in the Pacific and this presentation will report on one of its initiatives: to identify through interview and focus group discussions with resettled refugee and migrant communities within a high-income country (Australia) the barriers and enablers promoting or impeding access to healthcare services.

Results and Conclusions While clearly not representative of all migrants and refugee populations, the voices of the community members featured in this study do provide a snapshot of the health systems issues that face the growing number of ethnic and other minority populations residing in high-income countries around the globe. With the tremendous movement of people across borders in the contemporary world, fuelled by globalization and new and evolving communication and transport technologies, it is increasingly important for States to both listen and respond to their growing minority populations to improve evidence based policy, planning and resource allocation to better their universal health care systems, and progress the right to the highest attainable standard of health for all.

4.8.4 Health policy agendas and processes

P.4.8.4.001 (A)

Rights rhetoric in the post-2015 health development agenda: interrogating the role of the right to health in advancing global health equity

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Key policy institutions increasingly recognize the right to health as providing legal and ethical principles to formulate health goals to replace the Millennium Development Goals which expire in 2015. Accordingly right to health rhetoric is increasingly utilized in key policy papers in this domain. This paper explores the extent of this rhetoric, and interrogates the potential impact of this language on the outcomes and processes of the post-2015 health development agenda.
MATERIAL AND METHODS We explore the extent and usage of human rights and right to health language in key United Nations documents and resolutions in the post-2015 health agenda, including from the World Health Organization, the UN System Task Team on the Post-2015 UN Development Agenda, the Global Thematic Consultation on Health, and the UN General Assembly. We assess when and how rights language is used, and analyse the implications of this rhetoric for the outcomes and processes of global health policy.
RESULTS We found that UN institutions have significantly increased their use of rights rhetoric within the post-2015 agenda setting process, aided and buttressed by the advocacy and submissions of social actors. The use of rights language bodes well for an equity and social justice-oriented approach to devising and implementing the post-2015 health agenda, as the significant focus on participation partially suggests. However we note that the use of rights rhetoric may not extend into policy formulation itself, and that explicit reference to binding human rights commitments in the realm of goals and indicators remains lacking.
CONCLUSIONS We conclude that the emerging use of rights rhetoric augers well for a growing focus on equity and social justice in global health policy. We suggest that for this trend to consolidate and move to action, more explicit operationalization of rights standards and language beyond principled commitments is necessary.

P.4.8.4.002 (A)

Achieving the right to the highest attainable standard of health through decentralization in Kenya

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The aim of this paper is to identify specific core elements that are necessary to achieving the right to health and health care services in Kenya. In determining these core elements, this paper shall investigate whether the decentralization of health and health care services in Kenya is among the methods developing countries can utilise to achieve the right to health. The health related Millennium Development Goals are specific. The right to health is addressed through three MDGs; one, to reduce child mortality (MDG 3), two, to improve maternal health (MDG 4) and three, to combat HIV/AIDS, malaria and other diseases (MDG 5). These MDGs may soon be succeeded either by the goal of Universal Health Coverage (UHC), defined by the World Health Organization (WHO) as access to needed, good quality health services – promotion, prevention, treatment and rehabilitation – for everyone, without the risk of financial hardship as a result of having to pay to access these services, or by the proposed Sustainable Development Goals (SDG) as favoured by the states collectively referred to as BRICS. However, the process of achieving the right to health either through UHC or SDGs must encompass the demands of 'progressive realization' of the core elements of the right to health. Accordingly, this paper shall consider the following 'model' core elements in ensuring that Kenya is moving towards achieving the right to health; firstly; financing the right to health through decentralized taxation, secondly; the availability, accessibility, affordability and quality (AAAQ) of essential medicines and thirdly; the importance of health workers and their regulation administratively and financially.

P.4.8.4.003 (A)

Community voice in the global health policy agenda: the essential health needs of marginalized communities in Asia

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In a global development context where the ideologies of the privileged few have defined the way forward for the rest of the world for almost 15 years, greater global interaction is required in contributing to 'a decent life for all'. As a regional hub for Go4Health, an international consortium of academics and members of civil society assigned by the European Commission to aid the formulation of new health development goals (NHDGs), we believe it is imperative that a strong interaction exist between North and South in order for NHDGs, in their push toward universal health coverage, to truly address the health needs of people all over the world. Our qualitative study consults with marginalized communities across Asia whose health inequities can be attributed to the acronym PROGRESS. In gaining an understanding of their essential health needs as well as their understanding of their right to health entitlements we seek to give voice to those whose voices are neither locally nor globally heard – to ensure that their needs are addressed in the post-Millennium Development Goal (MDG) global health policy agenda. Consultations with communities thus far indicate a need for improved access to services as well as reliable and accountable health systems. Ensuring that effective, accessible and accountable health systems and services function across the world are a collective responsibility, whereby both national governments and international governments and organizations must come up with clear guidelines not only around health targets but how those targets will come about to ensure that everybody can enjoy good health. Such an outcome can only rise out of continuous and inclusive international dialogue which in turn will lead to the creation of health policies that are truly global and address the needs of all, including those who cannot yet enjoy the basic human right to health.

P.4.8.4.004 (A)

The MDGs revisited: from the Millennium Declaration to the post-2015 agenda

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INTRODUCTION At the dawn of the new millennium, the United Nations Millennium Declaration set out a unique global

framework for achieving a 'more peaceful, prosperous and just world'. This framework was condensed into eight Millennium Development Goals (MDGs), to be achieved by 2015. With <3 years to the deadline and the discussion of the post-2015 agenda in full swing, we examine the impact of the MDGs with respect to their indicators, to the underlying wider policy objectives and promises stated in the Millennium Declaration, as well as implications for future health-related development goals.
MATERIAL AND METHODS Policy documents, literature and databases related to the development and monitoring of the Millennium Declaration and the MDGs were analyzed concerning underlying values, policies, achievements, and perceived strengths and weaknesses of the conceptual MDG approach and its implementation.
RESULTS AND CONCLUSIONS While the Millennium Declaration highlighted freedom, equality, solidarity, tolerance, respect for nature, and shared responsibility as its core values, not all of these have been adequately reflected in the MDGs. Thus, assessing the achievements of the MDGs against their specific indicators was complemented by the analysis of progress achieved in reaching the underlying policy objectives. While progress towards achieving the health MDGs 4, 5, and 6 has been observed globally, this was uneven between countries, whereby the MDGs 4 (Child Health) and 5 (Maternal Health) will not be reached in many countries. There has been less effort to assess progress of the political development agenda. Key elements of the Millennium Declaration such as 'peace, security and disarmament', and 'human rights, democracy and good governance' have not been reflected in the MDGs. The countdown towards 2015 should be seen as an opportunity to re-focus the global development debate on these issues, including human rights, equity, community participation, and global governance.

P.4.8.4.005 (A)

Back to the future: what would the post-2015 Global Development Goals look like if we applied the same methods used to construct the Millennium Development Goals?

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INTRODUCTION The Millennium Development Goals (MDGs) have substantially driven the development agenda since 2001, though critics have argued the process used to decide on the goals was narrowly technical and not adequately consultative. With the post-2015 development goals now under consideration, the United Nations (UN) have sought to undertake a much broader consultative process, engaging a series of national and technical consultations to inform its High-Level Dialogue. This research seeks to apply the methods used in constructing the MDGs to formulate a set of goals, for comparison both with the MDGs and the evolving post-2015 development goals.
MATERIAL AND METHODS The researchers used the UN Department of Economic and Social Affairs to identify UN Summits and Conferences from 2001 to 2012, (http://www.un.org/en/development/desa/about/conferences.shtml) and to follow the process described by Vandemoortele, identifying measurable goals and targets from their recommendations, and collating these into a proposed set of post-2015 development goals.
RESULTS AND CONCLUSIONS The process yielded nine goals, with five goals duplicating current MDGs 2, 3, 6, 7 and 8. A further four new goals were identified, achieving food and water security, ensuring accessible health care systems, combating non-communicable disease, and improving communications and infrastructure development. Conspicuous by their absence were current MDGs 4 and 5, addressing child and maternal health, though arguably the 2014 twentieth anniversary of the International Conference on Population and Development will re-focus global attention on sexual and reproductive health. An extended examination of the MDG process suggests that the final technical exercise built on a decade of complex and highly politicised agenda setting, with the final list the result of high level negotiations and political trade-offs. Rather than use the past decade of UN summits as a resource from which to draw its goals, the current process is using them to initiate the dialogue, with the discourse already complex.

P.4.8.4.006 (A)

The global politics behind the formulation of the Millennium Development Goals: what do they tell us about the post-2015 process?

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INTRODUCTION The Millennium Development Goals (MDGs) have been of significance in shaping the development agenda in the early 21st Century and the complex global governance frameworks that evolved in response. Yet despite their potent role, and beside the academic contributions of Vandemoortele and Hulme, limited analysis exists around the politics behind the formulation of the world's so-called 'biggest promise'. The popular representation of the MDG process as a largely technical exercise, selecting goals from a decade of United Nations (UN) summits, fails to acknowledge the complex politics, trade-offs and negotiations that secured their now pivotal role. This analysis uses the available literature in the public domain to identify the major political plays influencing the development of the MDGs, and the impact of these on the current processes that surround the formulation of the post-MDG health and development agenda.
MATERIAL AND METHODS A scoping review (Levac et al. 2010) was used to locate material in both the published and grey literature on the formulation of the MDGs. Reference lists were analysed and a tracking process was undertaken to identify and include documents of further relevance. The collated literature was subject to thematic and discourse analysis.
RESULTS AND CONCLUSIONS Our analysis highlights the complex 'high' politics at play in the determination of the MDGs, with particular reference to the roles of key bilateral and multilateral agencies including the United Nations, Organisation for Economic Cooperation and Development's Development Assistance Committee, the World Bank, and International Monetary Fund. Our findings are of importance in understanding the challenges that have faced the MDG 8's Global Partnership for Development, and the implicit global political tensions emerging despite the current highly-structured UN consultative processes for the post-2015 Development Goals.

4.9 Training health workers and North South partnership

4.9.1 Educating health workers to face future challenges

P.4.9.1.001 (A)

The surgical training program: increasing surgical capacity in sierra leone by training medical doctors and community health officers in surgical and obstetric skills - progress report

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BACKGROUND Surgical and obstetrical emergencies are poorly addressed in Sierra Leone. Lack of human resources is a main contributing factor. There are obvious needs for innovative strategies to cope with the huge unmet need for surgery and obstetric care in rural parts of the country. In East-Africa there is experience and evidence that surgical task shifting can increase the surgical capacity. The objective of the study is to implement a surgical training program for Medical Doctors (MD) and Community Health Officers (CHO) to perform basic life saving surgery at district hospital level. This is done in cooperation with the Ministry of Health and Sanitation (MOHS).
METHODS Two students are enrolled every three months, with the first 6 months of basic training in Masanga Hospital followed by 6 months rotations in partner hospitals. After 2 years an exam is obtained followed by houseman ships partly in governmental hospitals. Curriculum is based on the WHO Integrated Management of Emergency and Essential Surgical Care.
RESULTS In the period between January 2011 and March 2013, 17 students were enrolled, 1 MD and 16 CHOs. Two students have passed the final exams and have on average participated in 558 surgeries each. All students have participated in 5511 surgical procedures either as observer (25.1%), assistant (40.2%) surgeon supervised (13.1%) or surgeon independent (21.6%). The three most performed procedures were inguinal hernia repair (30.3%), caesarian section (16.4%) and explorative laparotomy (6.2%).
CONCLUSION Short course training of MDs and CHOs are feasible in Sierra Leone and can contribute to address the unmet need for surgical and obstetrical care within a short timeframe. Good cooperation between MOHS and the NGO CapaCare towards common goals is essential.
KEYWORDS Human resources, surgery, task shifting, Surgical Training Program

P.4.9.1.002 (A)

Training courses for health care workers in low-resource countries: the experience in Morrumbene, Mozambique

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BACKGROUND An integrated multi-sectorial development project named 'Brescia for Mozambique' was initiated in the province of Inambane in 2009. The sanitary project component involved training of health care workers (HCW) from the sanitary district of Morrumbene, an area with 138 489 inhabitants and 10 public health care centers.
OBJECTIVES To evaluate the performance of training courses for HCW in the sanitary district of Morrumbene.
METHODS The courses aimed to improve the knowledge, attitudes and skills of HCW on maternal and child health and endemic infectious diseases. The course programs were decided with the local sanitary team. Anonymous pre and post tests regarding the course themes were applied and participants answered to a course evaluation questionnaire. Various didactic resources (computers projection, educative videos, mannequins) and diverse modes of teaching were used. Practical lessons were organized in wards and in the local laboratory. Printed materials in the form of handouts, manuals or printed slides were given. Gadgets were delivered to the participants (bags and T-shirts) as well as office supplies (pens, notebooks, pencils, eraser). At the end of each course a certificate of participation was given.
RESULTS From 2009 to 2012 five training courses were organized, for a total of 78 participants. Main characteristics

Table 1 Main characteristics and results of training course for health care workers of the sanitary district of Morrumbene, Mozambique

Course title	PMCT of HIV infection	Cinical and diagnostic aspects of TB	Essential newborn care 1	Complications of labour and delivery	Essentfal newborn care 2
Year	2009	2010	2011	2012	2012
Duration in days (h)	3 (15)	3 (15)	4 (24)	3 (20)	2 (12)
N participants	11	16	19	18	14
Pre-test average rating	6.72 (SD ± 0A9)	3.93 (SD ± 1.81)	4.70 (SD ± 1.37)	7.18 (SD ± 0.91)	7.29 (SD ± 1.14)
Post-test average rating	9.09 (SD ± 0.37)	6.25 (SD ± 31.61)	7.3 (SD ± 2.22)	8.41 (SD ± 0.63)	8.07 (SD ± 1.64)
P value (*t* test for paired Samples)	0.001	<0.001	0.001	<0.001	0.04
Overall course evaluation (%) good excellent	6 (42.9)* 8 (57.1)	13 (57.1) 3 (18 7)	5(27.8) 13 (72.2)	6 (33.3) 12 (66.7)	6 (42.9) 8 (57.1)

and results of training courses are summarized in the table. Statistically significant increase between pre/post test media was detected in the five courses performed. Courses were considered good or excellent by all HCW. Improvement of participants' skills on newborn resuscitation was observed during practical lessons with mannequins.
COMMENTS We observed good results regarding knowledge acquisition and improving skills on newborn resuscitation by the HCW who attended the courses. However, changing in attitudes is difficult to evaluate and hard to achieve, making necessary continuous supervision of activities and incentives to put into practice what was learned during the training courses.

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P.4.9.1.004 (A)

GELearning: an educational tool for the diffusion of the ecosystem approach in the prevention and control of the vector-borne diseases

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BRIEF INTRODUCTION The GELearning is an educational tool that simulates virtual environments and situations analogous to those faced by professionals in their daily practice. It differs from an educational game because GELearning not only contains recreational activities oriented toward the acquisition or verification of skills, but also includes the necessary content for the player to perform the required activities by learning content, acquiring skills and reinforcing attitudes.
MATERIALS AND METHODS As part of the Leadership in Ecosalud for the control and prevention of vector-borne diseases (ETV) in Latin America and the Caribbean, GELearning Ecosalud ETV (www.eduavance.com/GELearning/ECOETV) was designed to help create a critical mass that employs the ecosystem approach in ETV. GELeaning requires two hours of dedication and the avatar is the same sex as the participant and is identified by its name. It is divided into four stages: the first simulates the arrival of the player to a community confronted by an epidemiological risk based on the Ecohealth perspective; in the second, the player outlines the intervention strategy from the eco-systematic perspective; in the third, the methodologies for each of the approach components are designed; and the fourth evaluates the intervention and its scaling.
RESULTS AND CONCLUSIONS GELearning was designed and implemented by a team of multidisciplinary professionals: epidemiologists, eco-health specialists, teachers, instructional designers, graphic designers and web application developers. The richness in the composition enables the player to confront realistic challenges. Its virtual construction makes it excellent for public use because it derives from novel information and communication technologies that maximize the construction of knowledge and collaborative work from virtual spaces.

P.4.9.1.005 (A)

Transcultural supervision of medical residents in Africa

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INTRODUCTION International medicine and global health are enjoying important fad amongst medicine residents in western universities. In response to demands, numerous internships in developing countries are being offered to medical residents. To avoid having these internships become part of medical tourism it is necessary to have the appropriate professorial supervision. In some universities, teachers accompany students while others rely on the host country professorial structure. Both options have pros and cons but neither one seems to be superior. Over the last number of years, the idea of transcultural supervision (also referred to as cross-supervision) has evolved. This concept consists in sharing professorial resources with those of the host country to supervise all residents, whether they are from the visiting or the host country. The success of this approach requires a number of prerequisites. One only has to think of the preparation of professors and students prior to the internship regarding the knowledge of the culture, of the language, of the customs, and of the social and religious context. It is also necessary to prepare host teachers for the arrival of foreign students and teachers.
METHODOLOGY A transcultural supervision project was undertaken by the faculties of medicine of Sherbrooke (Canada) and Bamako (Mali). On September 26, 2012, during a production workshop, 21 professors (11 from Canada and 10 from Mali) met in Montreal, spent the day discussing the issues surrounding transcultural supervision. Discussions dealt mainly on the impediments to and the facilitation of transcultural supervision, on desirable behavior and attitudes for efficient supervision as well as on potential solutions to delicate transcultural supervision.
CONCLUSIONS Transcultural supervision of medical residents is efficient but needs a number of prerequisites.

P.4.9.1.006 (A)

The application of and satisfaction to the 'Changning family doctor centered service model' in community health centers in Changning District, Shanghai

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BACKGROUND As one of the pilot areas of the community health service reform in China, Changning District has developed a family doctor centered service model ¯C the ¡°Changning Model¡± in community health centers since 2010. The expected role of family doctors is to become the guardians of health of community people, and gatekeepers of social health insurance. This study aims to assess the capacity of family doctors in providing healthcare service and to understand the satisfaction of family doctors to the 'Changning model'.
METHODS Face to face interviews was conducted among all family doctors in the 10 community health centers using a semi-structured questionnaire.
RESULTS In total, 138 family doctors participated in the investigation. Of them, 78.7% had a degree of Bachelor of Medicine. All of them had the qualification of doctors including 77 general practitioners. 73.8% of them were attending physicians. Averagely, each family doctor served for 450 community people under a contract. The contents of service covered basic medical service, public health service, health education and chronic disease management. Family doctor services have covered all communities. Of the 138 family doctors, 79% reported that they were satisfied with the ¡°Changning model¡± for its family doctor centered mechanism. However, majority of the respondents felt overloaded (92.7%) and stressful (88.3%) under the new service model. More than

56% of the family doctors were unsatisfied with their salary. About 67% family doctors were not positive to their promotion and career development.
CONCLUSION The Changning Model has established a platform for integrated medical service with disease prevention and public health activities. With a well-qualified human resource, establish benchmarks to understand the demands for community health service, identify gaps between demands and supplies, and establish performance-based payment and promotion mechanism should be put into agenda.
KEYWORDS Family doctor, capacity, satisfaction

P.4.9.1.007 (A)
Evaluation on the effects of the 'Changning family doctor centered service model' in community health centers in Changning District, Shanghai

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BACKGROUND As one of the pilot areas of the community health service reform in China, Changning District has developed a family doctor centered service model in community health centers since 2010. The essential services in Changning Model are providing individualized health services and chronic disease managements to the contracted families, establishing fast path for up-level hospitals referral if necessary, prioritizing health service for vulnerable population and integrating medical service with public health activities. This study aims to understand the awareness of the Changning model in community people, and their willingness to sign the contract with family doctor, furthermore, to evaluate the satisfaction of contracted families to the family doctor centered service.
METHODS Stratified cluster sampling was applied in this survey. Self-reported questionnaire investigation was conducted for data collection.
RESULTS In total, 436 residents in Changning District were sampled and investigated. Of which 230 (52.75%) had signed a contract with family doctors. Of those not yet signed the contract, 85.0% knew the family doctor concept, and 75.4% were planning to sign the contract. Factors influencing the willingness of signing the contract were health status of family member (having chronic diseases), and the extent of trust with family doctors. The satisfaction of residents who had signed a contract on family doctor services was high, almost all (98%) residents felt the convenience in seeing the doctors, referring to up level hospitals, purchasing medicines and receiving medical advices and health information under the contracted service. The residents expected that their family doctors would spend more time on outpatient service.
CONCLUSION The Changning family doctor centered service model has been well acknowledged by the community residents. The next step should be focused on how to improve the service and provide quality assured service.
KEYWORDS Family doctors, community health service, China

4.10 Sanitation

P.4.10.001 (B)
The latrine ownership ladder: a responsive approach to addressing peri-urban sanitation challenges in Ghana

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INTRODUCTION Improved sanitation is an important development agenda in many low-income countries. The objective of this paper was to examine Ghana's environmental sanitation policies to assess their responsiveness to the peculiar challenges which confront the promotion of improved latrines in peri-urban communities and to recommend a policy intervention for preventing open-defecation in such communities.
MATERIAL AND METHODS Reviews of sanitation policy documents, to assess their response to common challenges which confront the promotion of improved latrines in peri-urban areas of low-income settings, was backed by observations.
RESULTS Unreliable water supply, poor physical planning, lack of formal recognition of land and housing rights, low household income, among others, are presented in reports and scientific literature as common developmental challenges of the poor peri-urban setting, which pose constraints on the promotion of improved latrines. Ghana's policy of banning public latrines in residential communities – other than commercial centres – is less sensitive to these challenges and likely to increase the incidence of open-defecation. We identify a latrine ownership ladder approach which encourages shared ownership regimes such as cotenant-shared, neighbourhood-shared and community-shared while keeping household ownership as the ultimate option as more responsive. Such an approach could be more responsive to the above challenges through sharing of resources and ownership rights.
CONCLUSIONS Ghana's Environmental Sanitation Policy does not make adequate provisions for enhancing the promotion of an open-defecation-free environment in the peri-urban setting. We argue for the adoption of a latrine ownership ladder approach as one of the policy interventions to prevent open-defection.

P.4.10.002 (B)
Monitoring and evaluation tools for sanitation: a comparative study of mobile phone and paper based sanitation survey approaches in the Dangme-West District of Ghana

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INTRODUCTION The 2010–2015 Joint Monitoring Programme Strategy requires sustainable and continued efforts to improve sanitation monitoring and evaluation (M&E) to help achieve the Millenium Development Goal 2015 targets. Challenges collecting reliable and timely data on actual sanitation practices in resource poor settings remain and there is a need for new, innovative M&E tools for gathering reliable sanitaion data. In Ghana, data collection on sanitation coverage is by labour intensive and costly person administered

methods. However, about 75% of the population has access to mobile phones, indicating that mobile phone based surveying is feasible. This study aims to compare the validity, reliability and cost effectiveness of using an SMS administered sanitation survey to an in-person survey in a peri-urban setting in the Dangme-West District of the Greater-Accra Region of Ghana.
METHODOLOGY Four-hundred and fifty eight adults with mobile phones were randomly selected from the community and randomly divided into two equal cohorts of 229 adults. By May 2013, one cohort will receive the SMS based sanitation survey four times a year while the other will received the person administered survey four times a year. Data collection is on-going and once complete the 'external' and 'face' validity of each survey method will be analyzed and compared. Further, correlation coefficients that measure the 'test-retest' and 'parallel' reliability of each survey method will be analysed. Cost-effectiveness will be evaluated through comparative cost modeling of each survey approach.
RESULTS A prospect cohort study has never been used to compare different survey delivery mechanisms within the sanitation sector. If the SMS based survey proves to be as valid and reliable as a traditional person delivered sanitation survey, it may create the possibility for timelier, more reliable and more cost effective sanitation reporting to the Joint Monitoring Programme. This poster presentation will highlight novel aspects of the research approach.

P.4.10.003 (B)
The sea is a mighty cleanser: a community view from Prampram, Ghana

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INTRODUCTION The sea is a source of livelihood and serves as a waste disposal site to individuals and governments. This paper explores local perceptions of the sea both as a cleanser and a waste sink in Prampram, a semi-urban community at the coast of the Guinean Golf about 30 km from Accra.
METHODS This study adopted an ethnographic approach to understanding community perceptions of the sea including participant observation in the homes of 17 households and visits to the beach to interact with community members. In addition, twenty key informants were interviewed.
RESULTS The fieldwork revealed that the sea is revered and seen by the community as one of their biggest asserts. It is a means for cleansing during the installation of new chiefs, purifying the land from bad omen and diseases, curing illnesses and treating infertility. It is also perceived to detoxify whatever is dumped in due to its high salt content. Its vastness, breeze and scenery make it very convenient for defecation; the waves serve as a WC cleaning the beach of all faeces. While it might seem as a paradox, the combination of the cleansing and waste sink characteristics rather makes it a valuable resource in the local community.
CONCLUSION The beach is the preferred option for defecation because it posses certain characteristics such as good ventilation which their present sanitation technologies lack. These specific characteristics should inform future toilet designs.

P.4.10.004 (B)
The Choleraphone

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INTRODUCTION Cholera epidemics have caused hundreds of thousands of cases and deaths in Bangladesh for centuries. However, the distribution and magnitude of cholera in non-hospitalized cases in Bangladesh is still largely unknown, since people simply cannot afford to see a doctor, and/or they treat the diarrhoea at home with ORS, avoiding the statistics. By developing a novel method for household surveillance of cholera the Choleraphone will detect diarrhoea cases that otherwise would have never been registered. The project is a collaboration between the University of Copenhagen, the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) and the University of Dhaka.
MATERIALS AND METHODS The methods are based on the concept of mHealth, which utilizes mobile phones in disease surveillance. The reporting system will be implemented in a cohort of 400 randomly selected households in Tongi, a low-income area in peri-urban Dhaka. Each household will be given a mobile phone and instructed how to report diarrheal episodes. A medical officer will visit the household after each report. Through the mobile reporting system, the project addresses the problem of diarrheal recall, which is optimal within 48 h, meaning that the household ideally should be interviewed every 2 days. The sensitivity and specificity of the method is evaluated through interviews with a randomly selected subset of households in the cohort.
CONCLUSION By visiting the households when they report a diarrheal incidence, the medical officer is expected to visit a household twice monthly based on an average of 25 episodes of diarrhoea per household per year. The Choleraphone will therefore cut down the household visits by 80%, from 15 to 3 per month (with one normal surveillance visit), not only saving project money but more importantly saving 6 h of interviews per month (30 min each) herby reducing research fatigue and time commitment from the households.

4.11 Methods in disease surveillance

P.4.11.001 (A)
Successful detection of a minor mumps outbreak using school absenteeism surveillance system in rural China

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BRIEF INTRODUCTION Infectious diseases outbreaks occurred frequently in schools, it is therefore of great importance to collect data about absenteeism, which has been popularly used for early detection of disease outbreaks in syndromic surveillance. We aimed to describe a successful detection case of a minor mumps outbreak using school absenteeism in rural China.
MATERIAL AND METHODS Using a web-based syndromic surveillance system (ISS) in rural China, 33 primary schools which totally covered about 22 090 children were under surveillance in Hubei Province. The numbers of and reasons for

absenteeism were daily collected and reported into ISS by teachers and the corresponding data were timely checked by researchers from ISS.
RESULTS On April 23rd 2013, 5:00 pm., when the CDC staffs checked the records in ISS, they found three absenteeisms for mumps within one class from Shayang Experimental Primary School. Then epidemiological investigation was conducted in the morning of April 24th. Shayang Experimental Primary School consisted of 2259 children. The primary case occurred on April 22nd and it was a boy (8 years old) in Grade 2, who was absent for painful swelling of the parotid glands and fever. Thereafter, other cases began to occur. From April 22nd to 25th, a total of 14 mumps cases was identified among pupils in this school with seven cases occurring in April 24th and six cases occurring within one class (Table 1). Control measures were taken after the epidemiological investigation. After April 25th, no new cases occurred within 10 days.
CONCLUSIONS This is a successful detection of a minor mumps outbreak using school absenteeism surveillance system in rural China. Measures were taken in the early stage of this minor outbreak and thus the epidemic of mumps was soon controlled. Further efforts are warranted to develop auto alarm ability of ISS using school absenteeism surveillance system.

Table 1 Distribution of school absenteeism for mumps during April 22nd–25th 2013 in Shayang Experimental Primary School

	Date				
Class	April 22nd	April 23rd	April 24th	April 25th	Total
Grade 1 Class 6	0	1	1	0	2
Grade 2 Class 3	1	0	0	0	1
Grade 2 Class 6	0	0	0	1	1
Grade 2 Class 7	1	1	3	1	6
Grade 4 Class 1	1	0	2	0	3
Grade 4 Class 3	0	0	1	0	1
Total	3	2	7	2	14

P.4.11.002 (A)

Evaluation of tongue colour as determined by Pantone CAPSURE as a good surrogate for oxygen saturation range

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INTRODUCTION Cyanosis is considered the most specific physical sign for hypoxaemia, however, the ability to detect cyanosis varies greatly among clinicians. Whilst it is recognised that the most optimal method of assessing arterial oxygenation is using pulse saturation oximeters, it is commonly unavailable in resource-scarce countries due to economic reasons. We introduce the use of Pantone CAPSURETM, a colour matcher, which is able to replicate colour and provide objective data for analysis. We aim to evaluate how well tongue colour can correlate to defined oxygen saturation (SpO2) ranges.
METHODS Forty-five participants with probable hypoxaemia and 24 healthy volunteers were recruited. Participant's tongue colour and concurrent SpO2 were measured using the colour matcher and pulse saturation oximeters respectively. Components of the colour, measured as breakdown percentages of red, green and blue (RGB) as well as cyan, magenta, yellow and key (CMYK) were recorded.
RESULTS Analysis showed that at higher SpO2, the percentage of red was higher ($r = 0.595$, $P < 0.001$) and colour intensity measured as the average of RGB ($r = 0.510$, $P < 0.001$) was higher. When data was divided into three levels according to SpO2 [Group 1: 89–92%, Group 2: 93–96% and Group 3: 97–100%], the mean were statistically different from each other ($P < 0.05$). In comparing individual named colours, mode analysis showed no significant difference ($x^2 = 102.554$, $P = 0.062$) between the three groups. However, mean colours for the low and high SpO2 groups showed that subjectively, substantial difference exists.
CONCLUSION This study has demonstrated proof of principle for the use of objective colour measurement to identify desaturation. The colour matched to low and high SpO2 ranges can be clearly differentiated. The regression line suggests that further research in patients with lower SpO2 would be helpful to take forward the hypothesis that colour matching can be used as an aid in the recognition of cyanosis in resource poor settings.

P.4.11.003 (A)

Quantitative analysis of the timeliness and completeness for data collection in a syndromic surveillance system

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INTRODUCTION Syndromic surveillance system has great advantages in the early detection of infectious diseases, especially in resource constraint settings. Its capability of early detecting for epidemics is impacted on the timeliness and completeness of data collection. Thus quantitative analysis of association between timeliness and completeness contribute to improvement for detection capability of surveillance system.
MATERIAL AND METHODS A web-based syndromic surveillance system (ISS) for infectious disease was established in rural China. Eighty health care units were included in ISS in Qianjiang County, Hubei Province. Doctors in charge of data collection were required to report the data into ISS daily. Using the data reported in ISS from April 1st 2012 to March 31st 2013, we calculated the Timely Reporting Proportion (TRP) and Data Integrity Rate (DIR) within required time of 1–7 days respectively. Then the fitting functions of dose-response associations of them were figured out.
RESULTS About 494 516 records from 80 health care units were reported in ISS system from April 1st, 2012 to March 31st, 2013. 96.67% of units have submitted the data as required. Among them, 80.91% of units submitted their daily records within the first day, accounting for 78.16% of total records. Both the TRP and DIR are increasing with the extension of required time, and tend to be stable after the 3rd or 4th day (about 95% for TRP and 90% for DIR) (Figures 1–2). Then fitting functions were generated to describe the association between them quantificationally (Figure 3).
CONCLUSIONS According to the fitting function of dose-response, the TRP should be improved up to 93% to approach the stable level of DIR. Although ISS syndromic surveillance system worked well with a high quality of data collection in

Qianjiang County, there is still promotion space for the TRP within the first three days.

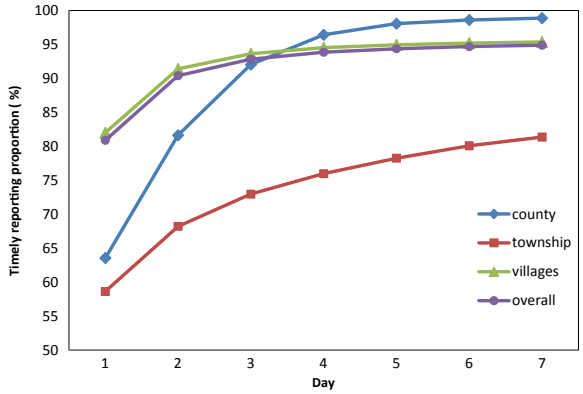

Figure 1 Timely reporting proportion within 1–7 days for each unit level. timely reporting proportion is shown as (Nr of timely reporting units/Nr of all units).

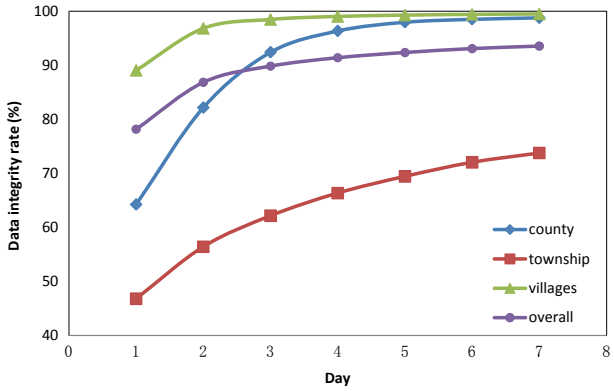

Figure 2 Data integrity rate within 1–7 days for each unit level. Data integerity rate is shown as (Nr of records reported timely/Nr of all records).

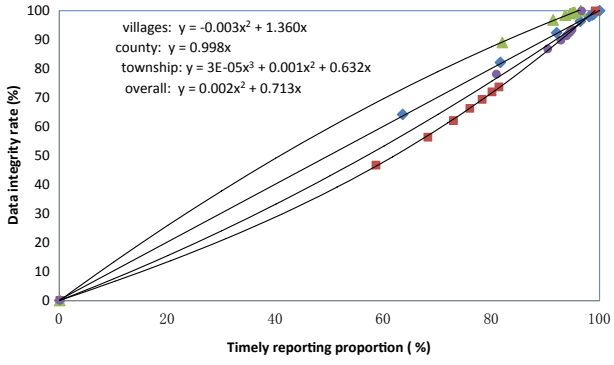

Figure 3 The dose-response associations of data integrity rate and timely reporting proportion for each unit level and their best fitting functions. Timely reporting proportion is shown as (Nr of timely reporting units/Nr of all units). Data integerity rate is shown as (Nr of records reported timely/Nr of all records).

Track 5: Global interaction and health

5.1 Travel medicine

P.5.1.001 (A)

Vaccinations for international travellers travelling from Greece

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INTRODUCTION To assess pre-travel vaccinations for international travellers who receive pre-travel advice in Greece. MATERIAL AND METHODS Prospective questionnaire-based survey of international travellers who received pre-travel advice at the Health Departments from 01/01/2009 to 31/12/2010. RESULTS A total of 2494 travellers were studied from 01/01/2009 to 31/12/2010. Sub-Saharan Africa was the most common destination (34.7%). Most travellers (60.8%) travelled for <1 month, for recreation (58.9%), stayed in hotels (65.3%), and in urban areas (53.6%). Yellow fever, tetravalent meningococcal, typhoid fever, cholera, and rabies vaccines were administered to 1629 (65.3%), 666 (26.7%), 615 (24.7%), 28 (1.1%), and 12 (0.5%) travellers, respectively. Of those who received Yellow fever vaccine, 737 (45.2%) and 332 (20.4%) travelled to sub-Saharan Africa and South America respectively; 949 (58.3%) stayed for short term, and 762 (46.8%) in urban areas; of all 1629 travellers, 150 (9.2%) and 226 (13.8%) travelled to areas of sub-Saharan Africa and South America respectively, where the vaccine is not generally recommended. Of those travellers who received meningococcal vaccine, 327 (49.1%) travelled to the Middle East for the Hajj and 251 (29%) to sub-Saharan Africa; 410 (61.6%) travelled for short term and 540 (64.4%) stayed in urban areas. Of those travellers who received typhoid vaccine, 241 (39.2%) and 78 (12.7%) travelled to sub-Saharan Africa and Indian subcontinent respectively; 234 (38%) travelled for short term, and 419 (68.1%) stayed in urban areas. CONCLUSIONS There is a need for more selective vaccine recommendations for travellers to developing countries, taking under consideration travellers' and travel characteristics as well. Strategies to target travel health consultants should be developed in order to increase awareness in travel health issues.

P.5.1.002 (A)

Comparative benefit of malaria chemoprophylaxis modelled in United Kingdom travellers

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INTRODUCTION Chemoprophylaxis against falciparum malaria is recommended for travellers from non-endemic countries to malarious destinations, but debate continues on benefit, especially with regard to mefloquine. Quantification of benefit for travellers from the United Kingdom (UK) was modeled to assist clinical and public health decision making.
MATERIAL AND METHODS The model was constructed utilizing: World Tourism Organization data showing total number of arrivals from the UK in countries with moderate or high malaria risk; data from a retrospective UK Clinical Practice Research Datalink (CPRD) drug utilization study; case fatality and drug utilization data from the travel medicine literature. Chemoprophylaxis with the following agents was considered: atovaquone-proguanil (AP), chloroquine with and without proguanil (C±P), doxycycline (Dx), mefloquine (Mq). The model was validated for the most recent year with temporally matched datasets for UK travel destinations and imported malaria (2007) against UK Health Protection Agency data on imported malaria.
RESULTS The median duration of chemoprophylactic use for each agent in CPRD was: AP 3.3 weeks, C±P 9 weeks, Dx 8 weeks, Mq 9: the maximum duration for all agents was 52 weeks. The model correctly predicted falciparum malaria deaths and gave a reasonable estimate of total cases – model: 5 deaths from 1501 cases; HPA: 5 deaths from 1153 cases. The number needed to take chemoprophylaxis (NNT) to prevent a case of malaria was 299 for Mq and 293 for the other three agents (AP, C±P, Dx) combined and assessed as a single group. The NNT to prevent a malaria death was 92528 for Mq and 90605 for AP, C±P, Dx combined.
CONCLUSIONS The model correctly predicted the number of malaria deaths and provides a robust and reliable estimate of the number of imported malaria cases in the UK. Protective benefit conferred by Mq was very similar to that of AP, C±P, Dx combined.

P.5.1.003 (A)

Vaccinations and malaria prophylaxis for long-term travellers travelling from Greece

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INTRODUCTION Long-term travel is defined typically as a travel of ≥1 month duration. Long-term travellers are at higher risk for acquisition of a wide range of infectious diseases compared to short-term travellers. The purpose of this study is to assess pre-travel vaccinations and malaria prophylaxis for long-term travellers who receive pre-travel advice in Greece.
METHODS AND MATERIALS The study was a prospective, questionnaire-based study which was carried out from January 1, 2009 through December 31, 2012 at 57 Regional Public Health Departments.
RESULTS A total of 4721 travellers were studied. Travellers sought pre-travel advice at a mean of 19.7 days (range: 0–349 days) before departure. Long-term travellers (≥1 month) accounted for 2205 (46.7%) of all travellers. Long-term travellers had a mean age of 34.5 years. The majority of them were men (79.8%). Sub-Saharan Africa was the most common destination (17.7%). Most long-term travellers pursued trips for work purposes (70%), visited urban areas (79.6%) and stayed in hotels (29.2%). Yellow fever, typhoid fever, hepatitis A and tetanus/diphtheria vaccines were administered to 1647 (74.7%), 741 (33.6%), 652 (29.5%), and 589 (26.7%) travellers, respectively. Yellow fever vaccine was administered to 339 (87%) and 132 (71%) long-term travellers to sub-Saharan Africa and South America respectively, whereas typhoid vaccine to 119 (90.8%) and 330 (84.6%) of those travelling to the Indian subcontinent and sub-Saharan Africa respectively. Malaria prophylaxis was recommended to 446 (20%) of long-term travellers. Mefloquine was the most commonly (49%) prescribed agent.
CONCLUSION Recommendations for vaccine and malaria prophylaxis for long-term travellers to developing countries should be more selective, based on the assessment of travellers' and travel characteristics, in order to provide adequate pre-travel preparation for this high risk group of travellers. Increasing awareness of travellers and travel health consultants is crucial.

P.5.1.004 (A)

A new guideline for malaria prophylaxis for travellers from Italy to endemic countries

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INTRODUCTION Malaria prophylaxis recommendations have been changing in the last few years, with a wide variability evident in official guidelines. WHO, CDC and French guidelines appear conservative, recommending chemoprophylaxis in most of endemic areas, while German speaking countries recommendations prefer emergency stand-by treatment (SBT) in the majority of settings. International experts do not agree about the use of chemoprophylaxis, and often do not adhere to guidelines. The Italian Society for Tropical Medicine (SIMET) set up a working group for updating national recommendations, involving other national scientific societies interested in this field.
MATERIAL AND METHODS A group of eight experts in travel medicine was established, and worked both on line and during live meetings in 2012. The basis of discussion were national and international guidelines, results of the opinion of international experts (Delphi method studies), evidence based data on the risk of malaria (from Malaria Atlas Project, WHO provided Annual Parasite Indexes-API, literature reporting malaria incidence in travelers).
RESULTS A comprehensive guideline for malaria prevention was written, reporting the role of insect bite prevention, chemoprophylaxis, stand-by emergency treatment, and early diagnosis. The main innovations are: the possibility of a first choice and a second choice for every geographic area, according to minor risk factors, the exclusion of chemoprophylaxis in *P. vivax* only areas, a graduation of medications for chemoprophylaxis according to drug resistance, previous experiences, tolerability. The recommendation of chemoprophylaxis as first choice in areas with API>10/1000 or risk in travelers >10/100.000 (Africa, East India, Myanmar, East Indonesia, Papua, inner Guyanas and Suriname); SBT is

recommended if API 1-10, or travelers' risk 1-10/100.000; only early diagnosis if indexes are below.
CONCLUSIONS The guideline will be distributed to specialists and general practitioners within the country, will be open for discussion via the SIMET web site, and will be updated in two years' time.

P.5.1.005 (A)

Mid-term follow-up study of 12 travelers with acute muscular Sarcocystis-like illness

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INTRODUCTION An outbreak of muscular Sarcocystis-like illness has been evidenced since summer 2011 among travelers after visiting Tioman Island, Malaysia. As of November 2012, GeoSentinel has been notified of 100 reported patients. A cluster of 12 patients returning since summer 2012 were seen in Bordeaux, France.
PATIENTS Cases were defined as travelers returning from Malaysia with persisting myalgia, unexplained blood eosinophilia, elevated CPK levels and negative trichinellosis serology.
RESULTS In September 2012, a cluster of 12 cases (seven male, five female; aged 11–46 years) were referred. The group was composed of four families. They spent their vacation together in August 2012 on the east coast of peninsular Malaysia. Of note, the three members of one family declined the optional 4-day stay on Tioman Island. Almost all patients presented high-grade fever, fatigue, headache, moderate-to-severe myalgia and arthralgia. Three cases experienced febrile illness for more than 14 days. A muscle biopsy from one patient revealed intense myositis, but no intramuscular cysts. Half of patients healed using usual antalgic treatments. Six patients were given empirically albendazole 400 mg b.i.d for 7 days and oral prednisone (0.5 mg/Kg/day) for 3 days tapered over 2 days. Six weeks later, four patients were still unrecovered. Prolonged manifestations were asthenia and myalgia. The patients were effectively treated with additional prednisone 0.5 mg/kg/day for 5 days and decreasing dosage over 14 days.
CONCLUSION The cluster of 12 diseased travelers mainly contributed to the 65 patients returning from Malaysia who represent the second wave of the outbreak. With little known about this foodborne anthropozoonosis, our experience suggests that late manifestations may occur and short term corticosteroid course could benefit symptomatic patients.

P.5.1.006 (B)

Vaccine requirements for Hajj – what's new?

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INTRODUCTION Each year approximately four million Muslims congregate in Makkah, Saudi Arabia, as part of the religious pilgrimage known as Hajj. This assembly of people from different parts of the world creates unique situation that may have global health impact. Spread of infectious and communicable disease has always been a concern in such a mass gathering.
METHODS The Saudi Arabian Ministry of Health, regional and international health agencies regularly update the vaccine requirement for Hajj. Current literature review was undertaken to determine the vaccine recommendations of pilgrims coming to Saudi Arabia. Epidemiological studies pertinent to Hajj were also reviewed to determine the source of infectious disease outbreak and possible control measures.
RESULTS All pilgrims coming to Saudi Arabia are required to take flu and meningococcal vaccine. Pilgrims coming from certain countries may require particular vaccine (e.g. Polio vaccine). Individual vaccine requirements are also considered keeping in view the medical and social condition of the pilgrim and their country of origin.
CONCLUSIONS It is imperative for all travel medicine practitioners and primary care providers to ensure that the intending pilgrims are up to date with the recommended vaccine requirements so as to minimize the chances of infectious disease spread and outbreak. This is particularly important as the medical community is embracing with possibility of new and emerging infections (such as Corona virus).

P.5.1.007 (A)

Ten years of Imported Malaria in Portugal – the burden of the disease and the comorbidities

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INTRODUCTION Although eradicated in Portugal, malaria keeps taking its toll on travellers and migrants from endemic countries, mainly Africa and Asia, all linked by extensive cultural and commercial relations. Malaria burden considering comorbidities, length of stay and lethality (2000–2009) is analysed.
MATERIAL AND METHODS Data, provided by the Central Administration of Health System (ACSS), refer to the national database of the diagnosis related groups (DRG). Between 1st January 2000 and 31st December 2009, 2003 discharges were registered in National Health Service hospitals with malaria diagnosis. For data selection ICD-9 CM were used, the codes were 084 (malaria) and 647.4 (malaria in the mother classifiable elsewhere, but complicating pregnancy, childbirth, or the puerperium). The variables analysed were: gender, age, length of stay (LOS), lethality and comorbidities. A descriptive analysis was carried out, using the usual indicators of frequency synthesis and hypothesis testing (t-tests, chi-square tests).
RESULTS There were 2003 malaria hospitalizations with decreasing annual frequency. Most cases were male (71%) aged 18–64 years (82.3%). In-hospital lethality was 1.95% and mean LOS 8.09 days. In those aged 18–64 years LOS and lethality were significantly higher ($P < 0.05$) and no differences were observed for gender. Those with HIV infection had significantly longer LOS ($P < 0.05$) but not higher lethality. Having pneumonia, respiratory failure or renal failure resulted in higher length of stay and higher lethality ($P < 0.05$). Having chronic bronchitis, diabetes, hypertension did not interfere with LOS or lethality, nor did malaria related to pregnancy. Those that died had a significantly longer LOS ($P < 0.05$).
CONCLUSIONS In ten years there were 2003 malaria hospitalizations in Portugal. Only 480 hospitalized cases were notified (national compulsory disease notification surveillance). LOS and lethality remained relatively stable in spite of decreasing malaria incidence. Older age and comorbidities, namely pneumonia, respiratory or renal failure, were associated with longer LOS and higher lethality.

P.5.1.009 (A)

Hepatitis A in immigrants returning home to visit friends and relatives in Barcelona (Spain)

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Immigrants Specially their children, returning to visit friends and relatives (VFRs) are at higher risk of developing Hepatitis A. Moreover, this group influences the epidemiology of Hepatitis A virus transmission in their country of residence.
Material and Methods We included in this study Hepatitis A cases in VFRs group observed from January 2006 to March 2013 in South Metropolitan area of Barcelona. The population of this area is about 700.000 inhabitants within 12% of immigrants. Serological diagnosis was made in the Laboratori Clínic L'Hospitalet (Catalan Institute of Health) by detection of IgM antibody to Hepatitis A virus using the chemiluminescent immunoassay (Vitros® Johnson & Johnson). Primary case is the first case in a defined outbreak or epidemic and secondary cases are patients who reported contact with primary case. Risk factors were analyzed according to the information collected by the Epidemiological Surveillance Unit.
Results There were 21 VFRs cases of Hepatitis A,15 men and six women, of whom 18 (85.7%) were aged less than 16 years old, age range was from 2 to 33 years. The countries visited were: Morocco (7), Ecuador (4), Peru (4), Bolivia (3), Pakistan (2) and India (1). During the study period, there were four outbreaks caused by primary cases from VFRs, 2 in household and two in school, both caused 13 secondary cases. One of the primary cases from school outbreak was asymptomatic.
Conclusions Among immigrant population, children who travel to visit friends and relatives are one of the main group at risk for developing hepatitis A in our city. To prevent disease and transmission in the country of residence this group should be vaccinated. Missed opportunities to prevent Hepatitis A should be addressed by vaccinating immigrants' children from endemic areas when they attend to primary care because rarely attended to pre-travel health care.

P.5.1.010 (A)

Imported malaria: epidemiological and clinical analysis of cases diagnosed in University General Hospital of Alicante (Spain) during 1995–2012

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Introduction Malaria is the main imported disease in Spain. Objective is to describe epidemiological and clinical pattern of imported malaria in the University General Hospital of Alicante (UGHA).
Material and Methods Retrospective review of clinical records of patients with malaria diagnosed between 1/1/1995 until 20/02/2012 in the UGHA.
Results One-hundred and nine cases of malaria, Age: 31.9 ± 15.5; pediatric cases 15.6%. Immigrants: 33.9%, european travelers: 28.4%, VFR 37.6%. 56.1% (60 cases) had history of previous malaria and the 91.7% (100 cases) came from Subsaharian Africa. 68.4% of the travelers/VFR not made any malaria chemoprophylaxis. Plasmodium falciparum was detected in 76.5% of the cases. Median time between clinical onset and diagnosis: 4 days. 81% of the patients was admitted to the hospital. Symptoms/signs more frequents: fever 96.3%, chills 75%, headhache 53.7%, swealling 51.9%, arthromyalgias 47.2%, splenomegaly 29.4%, vomits 28.7%, hepatomegaly 26.9% and diarrhoea 25.9%. More frequents laboratory alterations was: LDH >250 UI/l (89%), platelets counts <150 000/mm^3 (68.6%), AST >40 UI/l (62.5%), total bilirrubin >1 mg/dl (53%), haemoglobin <12 g/dl (42.9%), leukocytes counts <4000/mm^3 (26.7%). Mean of leukocytes counts (5242/mm^3 vs. 7062/mm^3; $P = 0.006$) and platelets counts (10258/mm^3 vs. 173267/mm^3; $P < 0.001$) was minor in travelers/VFR than in immigrants. Headache (61.4% vs. 39.5%; $P = 0.028$) was more frequent in travelers/VFR and enlarged spleen (21.4% vs. 43.6%, $P = 0.014$) in immigrants. Fifteen patients (13.7%) presented complications, without differences between travellers/VFR and immigrants. Evolution: cure in 107 cases (98.2%), dead in 1 case (0.93%), severe neurology saequeles in 1 case (0.93%) and vivax malaria relapse in 1 case.
Conclusions (i) Differences were observed in malaria clinical and biological pictures between travellers/VFR and immigrants. (ii) Clinical evolution is similar in travellers/VFR and immigrants. (iii) Its must be to remark on the importance of a correct compliance of malaria chemoprophylaxis in travellers/VFR.

P.5.1.011 (A)

Pediatric versus adult imported malaria: comparison of epidemiology, clinical and laboratory characteristics and outcome in a regional hospital setting in Barcelona (January 2009–March 2013)

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Introduction Malaria has increased in our non-endemic area due to increasing international travel and migration from endemic malaria countries. The aim is to compare epidemiology, clinical and laboratory characteristics and outcome of imported malaria among pediatric and adult population.
Material and Methods Observational retrospective study of all patients diagnosed of malaria from January2009 to March2013 at Barcelona hospital setting. Patients were classified as pediatric (0–17 years old) or adult (>17 years). For diagnoses we used thin blood smear, rapid diagnostic test.
Results We have reported 66 cases,19.7% were below 18 years. Mean age 31.7 years (0–53). 10.6% were Spanish and 89.4% immigrants, most cases from Senegal (57.6%). Were male 83.3%. *P. falciparum* (94%), *P. ovale* (3%), *P. vivax* (3%). Reason for travelling was Visiting Friends and Relatives (VFR) in 46.2% pediatric versus 84.9% adult. Mean days between arrival and consult was 10.9(range 0–30) in pediatric and 13.5(range 0–270) in adult. Started chemoprophylaxis 30.8% of pediatric but only 7.7% completed it, versus 11.3% adult who started it and 7.5% who finished it. Main clinical and laboratory characteristics in pediatric were fever (100%), vomiting (53.8%), weakness (61.5%), diarrhea (14.4%), abdominal pain (53.8%), anaemia (76.9%), thrombocytopenia (69.2), leukopenia (15.4%), hyponatremia (61.5%); and in adul twere fever (95.8%), vomiting (50%), weakness (69.7%), arthritic pain (52.8%), headache (67.9%), anaemia (24.5%), thrombocytopenia (88.7%), leukopenia (26.4%), hyponatremia (26.4%). Mean parasitemia in pediatric was 2.92% vs. 1.87 in adult. 23% of pediatrics had severe malaria and 15.4% required

ICU versus 5.7% severe malaria in adults and 1.9% required ICU.

CONCLUSIONS Most cases of imported malaria were by *P. falciparum* affecting young subsaharian male, travelling to Africa for VFR. Compare to adults, pediatrics consulted before, and were already on chemoprophylaxis, although not completed properly. Pediatrics have more abdominal pain, and anaemia. On the other hand, adult use to consult for more arthritic pain and headache and had more thrombocytopenia. Most cases of malaria were mild in both populations, although parasitemia was higher in pediatrics.

	Pediatric *n* = 13 (19.7%)	Adult *n* = 53 (80.3%)	Total *n* = 66 (100%)
Age (years)			
Mean	9.5	37.1	31.7
Range	0–17	23–53	0–53
Sex			
Female	5 (38.5)	6 (11.3)	11 (16.7)
Male	8 (61.5)	47 (88.7)	55 (83.3)
Nacionality			
Côte d'Ivoire	1 (7.7)	0 (0)	1 (1.5)
Spain	3 (23.1)	4 (7.6)	7 (10.6)
Gambia	3 (23.1)	4 (7.6)	7 (10.6)
Guinea Conakry	1 (7.7)	6 (11.4)	7 (10.6)
Senegal	5 (38.5)	33 (62.3)	38 (57.6)
Equatorial Guinea	0 (0)	1 (1.9)	1 (10.6)
Mali	0 (0)	5 (9.4)	5 (7.6)
Travel			
Arrival to Europe	6 (46.2)	1 (1.9)	7 (10.6)
Work	1 (7.7)	3 (5.7)	4 (6.1)
VFRs	6 (46.2)	45 (84.9)	51 (77.3)
Holidays	0 (0)	4 (7.5)	4 (6.1)
Days between arrival and consult			
Mean	10.9	13.5	12.9
Range	0–30	0–270	0–270
Chemoprophylaxis started	4 (30.8)	6 (11.3)	10 (15.2)
Chemoprophyaxis completed	1 (7.7)	4 (7.5)	5 (7.6)
Country visited			
Côte d'Ivoire	1 (7.1)	0 (0)	1 (1.3)
Gambia	5 (35.8)	4 (6.6)	9 (12.1)
Guinea Conakry	1 (7.1)	6 (9.8)	7 (9.3)
Equatorial Guinea	0 (0)	1 (1.6)	1 (1.3)
Senegal	7 (50.0)	35 (57.4)	42 (56.0)
Mali	0 (0)	7 (11.5)	7 (9.3)
Mauritania	0 (0)	2 (3.3)	2 (2.7)
India	0 (0)	2 (3.3)	2 (2.7)
Nicaragua	0 (0)	2 (3.3)	2 (2.7)
Panama	0 (0)	1 (1.6)	1 (1.3)
Costa Rica	0 (0)	1 (1.6)	1 (1.3)
Symptoms			
Fever	13 (100)	52 (98.1)	65 (98.5)
Vomiting	7 (53.8)	26 (49.1)	33 (50.0)
General weakness	8 (61.5)	38 (71.7)	46 (69.7)
Diarrhea	2 (15.4)	14 (26.4)	16 (24.2)
Abdominal pain	7 (53.8)	17 (32.1)	24 (36.4)
Arthritic pain	1 (7.7)	28 (52.8)	29 (43.9)
No symptoms	0 (0)	0 (0)	0 (0)
Headache	0 (0)	36 (67.9)	36 (62.1)
Seizures	0 (0)	1 (1.9)	1 (1.5)
Hematuria	3 (23.1)	2 (3.8)	5 (7.6)
Physical examination			
Hepatomegaly	1 (7.7)	7 (13.2)	8 (13.6)
Splenomegaly	1 (7.7)	4 (7.5)	5 (7.6)
Hepato-splenomegaly	3 (23.1)	6 (11.3)	9 (13.4)
Skin lesions	0 (0)	1 (1.9)	1 (1.5)
Jaundice	4 (30.8)	10 (18.9)	14 (21.2)
Temperature (ºC)			
Mean	38.1	38.2	38.15
Range	35.6–39.5	35.9–40.5	35.6–40.5
Pregnancy			
Laboratory	0 (0)	2 (3.8)	2 (3.0)
Anaemia (Hb < 12 mg/dl)	10 (76.9)	13 (24.5)	23 (34.8)
Thrombocytopenia	9 (69.2)	47 (88.7)	56 (84.9)
Leukopenia	2 (15.4)	14 (26.4)	16 (24.2)
Hyponatremia	8 (61.5)	14 (26.4)	22 (33.3)
Renal failure	2 (15.4)	2 (3.8)	4 (6.1)
High transaminases	0 (0)	9 (17.0)	9 (13.6)
Diagnostic			
Thin blood smear	13 (100)	53 (100)	66 (100)
Rapid Diagnostic Test	10 (76.9)	37 (69.8)	47 (71.2)
PCR	0 (0)	2 (3.8)	2 (3.0)
Specie			
P. faciparum	12 (92.3)	49 (92.5)	61 (92.5)
P. falciparum + *P. Malariae*	1 (7.7)	0 (0)	1 (1.5)
P. ovale	0 (0)	2 (3.8)	2 (3.0)
P. vivax	0 (0)	2 (3.8)	2 (3.0)
Plasmodium parasitemia (%)			
Mean	2.92	1.87	2.06
Range	0.01–13	0.1–14	0.01–14
Severe malaria	3 (23.0)	3 (5.7)	5 (7.6)
Required ICU	2 (15.4)	1 (1.9)	3 (4.5)
Co-morbility			
HIV	0 (0)	2 (3.8)	2 (3.0)
VHC	0 (0)	1 (1.9)	1 (1.5)
HbsAg	0 (0)	4 (7.5)	4 (6.1)

Table (*Continued*)

P.5.1.012 (A)

Drug utilization study of malaria chemoprophylaxis regimens

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Background Chemoprophylaxis is essential for travellers to malaria endemic regions. Differences in indications and recommendations exist for the different regimens. We aimed to assess the prescribing patterns for mefloquine and other antimalarials for chemoprophylaxis under real-world conditions.
Methods We conducted a descriptive drug utilization study using the U.K. Clinical Practice Research Datalink (CPRD). We assessed characteristics of individuals with a first-time antimalarial prescription for mefloquine (MQ), atovaquone/proguanil (AP), chloroquine and/or proguanil (C/P), or doxycycline (DX) between 2001 and 2012.
Results Of 165 218 individuals who were prescribed an antimalarial, 108 344 were prescribed AP, 25 294 MQ, 23 195 DX, and 8385 C/P. Between 99.7% and 99.9% of prescriptions were recorded for prophylactic use. The mean duration (in weeks) of prescription for chemoprophylaxis was 12.3 for MQ, 12.1 for C/P, 10.3 for DX, and 3.5 for AP. A total of 19.4% MQ prescriptions were for children younger than 12 years (compared to C/P 15.8%, AP 7.4%, and DX 0.4%). A total of 1.3% MQ prescriptions were for pregnant or postpartum females (compared to C/P 1.4%, DX 0.5%, and AP 0.4%). The majority of general practitioners (GPs) prescribing MQ had extensive experience in travel medicine (59.1%), whereas 17.5% of GPs prescribing C/P were less experienced in travel medicine. A large percentage (32.9%) of GPs prescribing MQ worked in practices in London.
Conclusions The prescription of antimalarials was in line with UK recommendations for malaria chemoprophylaxis. MQ was prescribed more frequently than other antimalarials for children and as frequently as C/P for pregnant women. These represent populations with a particular need for safe and effective chemoprophylaxis, given the increased risk of severe and complicated malaria in this group. Most practitioners prescribing antimalarials were experienced in travel medicine and observed contraindications.

P.5.1.013 (A)

Impact of land use on risks of vector-borne diseases in a global outreach tourist setting: a case study of Koh Chang, Thailand

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Introduction Dengue and malaria are major international public health problems. Tourists are reported to have been infected by these two diseases and to have brought dengue and malaria from tourist areas back into their home countries. Tourism development causes changes in land use which in turn impacts disease transmission. This study aims to investigate the relationship between disease incidence and change in land use on Koh Chang, a popular global outreach tourist destination in Thailand.
Materials and Methods Reported dengue and malaria cases from 2008 to 2010 on Koh Chang were analyzed along with changes in land use within a buffer zone of 500 meters from village locations by using GIS technology. The villages were separated into the East and West sides of the island based on differences in the intensity of tourism activities.
Results and Conclusions A total of 65 dengue and 39 malaria reported cases (2008–2010) were investigated. Dengue (90.76%; 15.84 per 1000 pop.) seemed to occur in the West, where the island is mostly populated with a high amount of tourism activities. Malaria (51.28%; 6.07 per 1000 population) was slightly more present in the East, where plantations predominate and where fewer tourism activities occur. Overall, dengue incidence increased (+83%; 6.24–11.39 per 1000 population; 2008–2010), whereas malaria incidence decreased (−43%; 7.12–4.09 per 1000 population; 2008–2010). In conclusion, change in land use had an impact on both dengue (increased incidence in human settlements) and malaria transmission (decreased incidence in human settlements/mixed orchards and coconut plantations). Our results provide evidences that will assist health authorities in making effective decisions regarding the surveillance and prevention of emerging infectious diseases on the tourist island.

5.2 Global interaction and health

P.5.2.001 (B)

Seroprevalence of trypanosoma cruzi (*T. cruzi*) infection in immigrants from Latin America in the Alicante Municipality

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Introduction In Valencian Comunity screening of imported Chagas disease (ChD) is currently performed in pregnant women and blood and organ donors from endemic areas, but there is no current legislation that includes screening for ChD in general population of Latin American origin. Objectives: 1. To estimate prevalence of *T. cruzi* infection (TCI) in Latin American population living in Alicante. 2. To study epidemiological variables associated with TCI.
Materials and Methods Observational, cross-sectional (prevalence survey) study through active case finding in social and recreational activities of Latin American community. All participants filled an epidemiological and knowledge questionnaire about ChD, were advised about early detection and finally offered them immunochromatographic rapid test (Chagas Ab Rapid ®, Standard Diagnostics, In.). Positive cases were confirmed by IFA and ELISA. Study Period: November 2011–May 2012.
Results Questionnaire was made to 347 people: 57% female, median age 37 years; major countries of origin: Bolivia (49%), Ecuador (21, 3%), Colombia (17.3%), Paraguay (3.7%) (3.7%) and Argentina (3.3%). Chagas rapid test were performed in 300 people (86.5%), of which 22 were positive (TCI prevalence 7.33%, 95%CI 4.2–10.4); 77.3% were women. Prevalence in bolivian subgroup was 12.93% (95% CI 7.5–18.35). In

multivariate analysis independently associated variables with TCI were: 'Bolivian origin' (OR 7.9, 95% CI 2.1–29, 5, $P = 0.002$) and 'had lived in mud house' (OR: 2, 4, 95% CI 0.97–6.23, $P = 0.056$).
CONCLUSIONS (i) The prevalence of TCI was relatively high (7.3%) compared with other series published in our country, (ii) As in other studies, the highest prevalence occurs among Bolivians (12.93%). (iii) The association of TCI with a history of residence in poor housing is significant. (iv) It is important to promote health programs or strategies that include active search for ChD to prevent chronic complications.

P.5.2.002 (B)

Imported infectious diseases in immigrants living at shelter centres or temporary assisted houses in Barcelona (Spain)

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BACKGROUND Immigrants may carry infectious disease as a result of diseases prevalence in their country of origin, exposures and conditions during migration. To facilitate cultural, social and work integration in the host country for recently arrived immigrants, public and private institutions created shelter centres for <18 years old and temporary assisted houses for >18 years old immigrants at risk of social exclusion.
MATERIALS AND METHOD This study is a retrospective review of imported infectious disease diagnosed in immigrants, living in shelter centres or temporary assisted houses, attended in one centre for imported diseases in Barcelona from January 2009 to December 2012.
RESULT A total of 206 cases were collected. Median age was 17.7 (11.0–49.3) years, 204 (98.6%) were male, 180 (87.0%) from sub-Saharan Africa. Median time of arrival was 4.6 (0.3–113.8) months. Clinically 141 (68.1%) were asymptomatic. In 140 (67.6%) at least one imported disease was diagnosed: 95 (53.1%) latent tuberculosis infection; 25 (12.9%) HBsAg positive, 4 (2.1%)%) anti-HCV positive, 9 (4.8%) syphilis serology positive, 2 (1.0%) HIV positive and 2 (1.0%) malaria cases. In blood test, 40 (22.3%) had eosinophilia >500 eosinophil/mm^3 and 46 (27.5%) IgE >500 UI/ml. In 62 (31.6%) at least one parasite with known relation with eosinophilia and/or hyper-IgE (PKREH) was diagnosed. The most common PKREH were: 20 hookworm, 20 schistotosmiasis, 11 giardiasis, 10 strongyloidiasis and 7 mansonelliasis. Statistical data showed a significant association between eosinophilia and/or hyper-IgE and presence of parasites ($\div 2$, $P < 0.05$). The study of eosinophilia and/or hyper-IgE was not completed in 24 (11.9%).
CONCLUSION The majority of immigrants were asymptomatic, but most of them had important infectious disease. These results support the idea that screening protocols are important in new arrived immigrants. There was a significant relation between eosinophilia and/or hyper-IgE and presence of parasites, so parasitological studies are extremely important in this group.

P.5.2.003 (B)

Results for the implementation of a screening programme for preventing congenital transmission of Chagas disease

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INTRODUCTION In Catalonia (Spain) we estimate around 156 000 women of childbearing age who originated from Chagas disease-endemic countries. In 2010, the Catalonian Health Department implemented a screening programme protocol of Chagas disease in Latin American pregnant women. This study evaluates the results of the first year of the implementation of the screening programme.
MATERIAL AND METHODS In 2010, pregnant women from Latin America, who received prenatal care in the public health system of Barcelona's southern metropolitan area, were tested in the Laboratori Clinic L'Hospitalet of the Catalan Institute of Health for the presence of *T. cruzi* antibodies. The laboratory diagnosis of Chagas disease was based on two serological tests. A recombinant EIA (Bioelisa Chagas Biokit®) was used as screening assay. Positive samples were confirmed by a native EIA (Ortho® *T. cruzi* System). Congenital infection was diagnosed by direct microscopic examination of microhaematocrit in the newborn blood and/or serological test performed at 9 months old.
RESULTS During the study period there were 1122 newborns notified to mothers from Chagas endemic areas, among them 233 were from Bolivia. Screening was performed to 882 pregnant women, 18 (2%) were positive and all were Bolivian. During the follow-up there were 3 abortions of which one was spontaneous and two women came back to their country of origin being all considered as lost to follow-up. Newborns from remaining thirteen women were tested and one was positive. However, four of them were lost to follow-up for serology at 9 months old. The vertical transmission rate was 5.6% (1/13).
CONCLUSIONS This preliminary data confirms the presence of transmission of Chagas disease in non endemic areas. Moreover, our findings highlights the relevance of implementing a screening programme and the importance of improving the follow-up on newborns from infected mothers.

P.5.2.004 (B)

Utilization of hospital services in Alicante (Spain): a comparative Analysis of foreign citizens from high-income countries, immigrants from low-income countries and Spaniards citizens

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INTRODUCTION The number of foreign residents in Spain has increased. There is no doubt that the health of the immigrant population has become a relevant subject from the point of view of public healthcare. Our study aimed at describing the diagnoses and service of admission in foreign citizens from high-income countries (FCHICs), foreign citizens from low-income countries (FCLICs) and Spanish-born.
MATERIAL Cross-sectional study in two hospitals of a public hospital in the city of Alicante and surrounding area (Spain). A multivariate analysis adjusting for age and sex was performed by

service of hospital admission and diagnosis [adjusted odds ratio (AOR) with 95% of confidence intervals (CI)].
RESULTS About 43 147 patients >15 year discharged from hospitals. The median of age of FCLICs (37 year) was lower than autochthonous citizens (64 years) and FCHICs (64 years) ($P < 0.001$). The gender of FCLICs was 66% female, in FCHICs 45% were female, and in autochthonous group 52% were females ($P < 0.001$). The FCHICs had more risk being diagnosis at discharges in the categories of circulatory system (AOR: 1.55; 95%CI: 1.35–1.77), neoplasms (AOR: 1.21; 95%CI: 1.03–1.42), injury and poisoning (AOR: 1.33; 95%CI: 1.11–1.58). The FCLICs had more risk of being diagnosis in the categories pregnancy, childbirth & puerperium (AOR: 1.33; 95%CI: 1.29–1.59), injury and poisoning (AOR: 1.19; 95%CI: 1.03–1.36), and had less risk in the categories mental disorder (AOR: 0.32, 95%CI 0.22–0.45). The FCHICs had more risk of being discharged from surgery (AOR: 1.24, 95%CI: 1.01–1.23) and less risk from traumatology (AOR: 0.88, 95%CI: 0.59–0.95). The FCLICs had more risk of being discharges from gynecology compared with autochthonous (AOR: 1.46; 95%CI: 1.31–1.62) had less risk from medical specialties (AOR: 0.79; 95%CI: 0.73–0.87).
CONCLUSION The profile of hospitalization in FCHICs was similar than autochthonous and the profile of hospitalization in foreign citizens from FCLICs was different compared autochthonous.

P.5.2.005 (B)

Prevalence of Chagas disease in the bolivian community in Palma de Mallorca, Spain

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OBJECTIVES To estimate the seroprevalence of Chagas disease in Bolivian residents in Mallorca, Spain, Secondary objetives: To compare risk factors, to know about the underdiagnosis of Chagas disease in the bolivian population.
MATERIAL AND METHODS Study: descriptive cross-sectional. Area: Health Area in Palma. Inclusion criteria: bolivian patients older then 18 years old, from two Primary health centres. Selection: systematic random sampling health card database, contacted by telephone. Exclusion criteria: physical or mental disability, impossibility of contact by phone call and who refused to participate or to sign the informed consent. Sample size and selection: From an estimated prevalence of 7%, a 3% accuracy and a confidence level of 95% and 30% of losses, 344 subjects were required. Measurements: In consultation with oral interview, and multiple choice to know the risk factors. Blood test. Cases were confirmed after two positive ELISA tests.
RESULTS We obtain a prevelance of 19.1%. Forty-eight positives cases of the total (251) subjects included. 57.8% were men. Mean age 34.6 years (SD = 9.3). We observed a higher prevalence in those who had lived in rural areas, and subjects coming from Cochabamba (50%) and Santa Cruz (25%). in houses of adobe, and those who had a family history of Chagas. All positive cases were confirmed by two different techniques (no false positives).
CONCLUSION We observed a high prevalence in the bolivian population in Palma de Mallorca, and specially in those with the more usual risk factors for this disease. It should be considered to screen for Chagas disease on this inmigrant population to prevent progression and to include it on the guidelines of our health system.

P.5.2.006 (B)

The Mecca pilgrimage and its medical preparedness

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BRIEF INTRODUCTION The pilgrimage to Mecca (Hajj) in Saudi Arabia, is an annual mass gathering which attracts over 2.5 million pilgrims, who must perform the Hajj at least once in their lifetime. It is the most important of all Muslim pilgrimages and one of the largest, culturally and geographically diverse, mass gatherings in the world. Because of its moving target date, since it follows the Islamic lunar calendar, and the volume of its attendance, it presents a unique and important public-health challenge, demanding constant preparedness of the host country. Travelers from over 140 countries are gathered during the Hajj, performing demanding rituals in highly overcrowded conditions.
MATERIALS AND METHODS The terms 'hajj', 'mecca', 'pilgrimage' and 'preparedness' were used in a search through PubMed, MEDLINE and Google Scholar. Articles were chosen from 1995 to 2012, with the majority of them being written in the last ten years.
RESULTS Many infectious and non-communicable diseases as well as other health hazards, are faced by pilgrims in the strenuous experience of the Hajj: Meningococcal meningitis, hepatitis B, hepatitis C, diarrhea, respiratory diseases, hemorrhagic fevers, stampedes and traumas etc.
CONCLUSIONS The Hajj presents a significant challenge that impacts the international public health as a very large number of humans move through continents to arrive to Mecca, with everything this entails in terms of potential risks in disease transmission and other health hazards. The preparations of the Saudi Arabia authorities in connection with international organizations concerning this mass gathering are crucial for the managing of the Hajj and the successful outcome of the event.

P.5.2.007 (B)

Sociodemographic factors and epidemiology of chagas disease in Bolivian population in Mallorca

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OBJECTIVES Main objetives: knowing sociodemographic factors of Chagas Disease in Bolivian community in Mallorca, Spain. Secondary Objetives: Identify the level knowledge and te presence of symptoms of Chagas disease in Bolivian population and estimate the prevalence of the disease.
MATERIAL AND METHODS Descriptive cross-sectional study in Mallorca Health Area. Inclusion criteria: bolivian patients >18 years old, assigned to two basic health areas. Selected by systematic random sampling health card database and were recruited bu telephone. Exclusion criteria: people with great physical or mental disability that precludes the studiy, those not having a phone and people who refuse to participate in the study. Measurements: In consultation we conducted a test of 38 questions covering topics:sociodemographic variables, risk factors, epidemiology of the disease, and presence of any symptoms related. Two serological Tests to consider a positive case.
RESULTS Two-hundred and fifty one subjects, 48 positive, prevalence of 19.1%. Most of the risk factors studied were significantly associated with presence of Chagas and most cases

reported have lived in rural areas, adobe houses, mother or family had affection and/or had visualized the insect. No differences by gender, years out of Bolivia or transfusion. Population have a good level of awareness of chagas, and thtat 75–90% responded correctly regardless of the results of serology. No significant differences regarding the presence of symptoms in positive patients, in part because symptoms can be nonspecific, difficult to value or enrolled in an asymptomatic phase of the disease.
CONCLUSION We found a high prevalence of Chagas disease in, in turn found a clear relationship with the presence of one or several risk factors, however there was no detection of a characteristic symptom. Overall the Bolivian population has a good level of knowledge about the disease.

P.5.2.008 (B)

Sensitivity and specificity of the Chagas Detect™ Plus Rapid Test (InBiOS International, Inc.) for the serological screening of Chagas disease using serum samples

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INTRODUCTION In Spain, Trypanosoma cruzi infection is an imported parasitic disease due to immigration from Latin America. As most of infected people are in the chronic phase of the disease, the diagnosis is mainly carried out by serological tests. The Chagas Detect™ Plus Rapid Test (InBiOS International, Inc., USA) is a new immunochromatographic test for the qualitative detection of anti-*T. cruzi* antibodies. The aim of this study was to compare the sensitivity and specificity of this test using well-characterized serum samples.
MATERIAL AND METHODS Well characterized serum samples from chagasic patients (CH, $n = 40$), non-chagasic individuals (NoCH, $n = 40$), visceral leishmaniasis patients (VL, $n = 10$) and malaria patients (Mal, $n = 10$) were included. The concordance of the results of two conventional tests (ELISA and IFAT) was considered as the gold standard.
RESULTS All samples from CH patients were detected by the rapid test, just one out of them showed a faint reaction. Two samples from NoCH individuals showed a faint reaction. Cross-reactivity with samples from VL patients was not found and one false-positive result was observed in samples from malaria patients. The sensitivity and specificity of the Chagas Detect™ Plus Rapid Test were estimated in two scenarios. Without problem in the interpretation of results as the first scenario and assuming that the faint reaction could not be detected as the second scenario. Taking into account these situations, the sensitivity was between 100% and 97.5% and the specificity was from 95% to 98.3, respectively.
CONCLUSION The Chagas Detect™ Plus Rapid Test had a promising performance for serological screening of anti-*T. cruzi* antibodies. However, the subjectivity in the interpretation of results could compromise the sensitivity of the assay. New studies with a large number of samples and different situations were necessary in order to know the real performance characteristics of this new rapid test.

P.5.2.009 (B)

Screening for imported infectious diseases in asymptomatic Sub-Saharan and Latin American immigrants at a tropical medicine unit in Spain

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INTRODUCTION Migrants may be asymptomatic and yet have infectious diseases acquired in their country of origin. Identifying and treating these patients could have an important impact both for the individual concerned and for public health.
METHODS Observational, comparative and retrospective study of asymptomatic sub-Saharan (SSA) and Latin American (LA) immigrants (ASYM-IMM) attended at the Tropical Medicine Unit, Ramón y Cajal Hospital (Madrid) (January 2000– January 2011) for a medical exam. Demographic variables: age, gender, country and area of origin and time from arrival to consultation (TtoC). Screening performed included: serum biochemistry, blood count, basic urine analysis, HIV, HBV, HCV, rapid plasma reagin (RPR) serological analysis, Mantoux skin test (PPD), stool parasites, PCR for malaria in SSA, *T. cruzi* serology for LA and schistosomiasis serology if risk factors.
RESULTS Three hundred and seventeen SSA. Median age: 29 years (IQR: 21–32) Median TtoC: 5 months (IQR2-10). Diagnoses: -PPD+: 71%; -Chronic HBV: 14%; -Eosinophilia: 13.2%; -Schistosomiasis: 5.9%; -Malaria: 4.6%; -HIV: 2.3%; -RPR+: 2.3%; -Chronic HCV: 1.3%; -Intestinal parasites: 2.9%. 383 LA. Median age: 32 years (IQR: 25–41) Median TtoC: 42 months (IQR: 25–62). Diagnoses: -*T. cruzi* +: 48.5%; -PPD+: 32.1%. -Eosinophilia: 13.8%; -HIV: 0.3%; -Chronic HBV: 1.6%; -Intestinal parasites: 1.5%; -RPR+: 1.4%; -Chronic HCV: 0%. -Schistosomiasis: 0%. Other relevant findings: 73% SSA had at least one positive HBV biomarker; three cases of *P. falciparum* diagnosed >1 year after arrival in SSA; most intestinal parasites diagnosed <1 year after arrival. Among those with eosinophilia ($n = 95$): prevalence of Strongyloides by stool analysis was 1.2% and by serology 56.1%.
CONCLUSIONS Proposed screening for infectious diseases in ASYM-IMM: serum biochemistry, blood count, basic urine analysis, HIV, HBV, HCV (in LA only if risk factors), RPR, PPD, Strongyloides serology, schistosomiasis serology (in LA only if risk factors); PCR for malaria (in SSA) and *T. cruzi* serology (in LA). Stool analysis if ≤6–12 months since arrival and/or eosinophilia.

5.5 Occupational and Environmental Health in a Globalized world

P.5.5.001 (B)

Evaluation of the estrogenic and hepatic effects of bisphenol-a contents in five selected canned foods on adult albino rats

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Concerns have been raised regarding the safety of human exposure to Bisphenol A (BPA), an unstable chemical capable of leaching from polycarbonate plastics products into foods and beverages. The study was undertaken to evaluate the effects of exposure of rats to different concentrations of Bisphenol A (0.6–4.1 μg/l) (concentrations detected in selected canned foods) ana-

lyzed in this study. BPA concentrations were determined in samples by gas chromatography and mass spectrometry. Thirty male and female rats (60–80 g) divided into six groups of five rats each were then administered standard BPA in the range detected in analyzed canned foods namely baked beans, sweet corn, tomato paste, green peas and sardine obtained from Markets in Lagos area, Nigeria, orally for seven weeks at different concentrations ranging from (0.6–4.1 μg/l). Hormonal and biochemical assays were carried out; ovary, testis and liver were excised for histological studies. Data were analyzed using ANOVA. The results showed that BPA had no significant effect on serum level of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in all the groups while progesterone, estradiol, and testosterone were significantly increased ($P \leq 0.05$) when compared to control groups. High dose of BPA (4.1 μg/kg) significantly increased ($P \leq 0.05$) levels of alkaline phosphatase (ALP) while there was no significant difference ($P \geq 0.05$) in the serum aspartate amino transferase (AST) alanine amino transferase (ALT) and bilirubin when compared with the control groups. Histological examinations of the test groups showed no difference when compared to the control but the ovaries had increase in the production of follicle as the doses of BPA increased. This study indicates that Bisphenol A has an induction effect on ovarian steroidogenesis. The exposure to bisphenol A in consumer products may have endocrine disrupting actions which could potentiate onset of carcinogenic reactions. Its use therefore should be replaced with suitable alternatives.

Track 6: 5th Conference of the Scandinavian-Baltic Society for Parasitology

6.1 Tick-borne diseases

P.6.1.001 (A)

Identification of novel humane pathogens in Danish ticks

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Tick-borne diseases are the most common vector-borne illnesses in the world. Ticks can carry and transmit bacteria, viruses, or parasites. Reports of tick-borne diseases worldwide are increasing. In Denmark, ticks collected from dogs in Southern and Western Jutland and South Fyn were examined for Borrelia burgorferi, Franciella tularensis, Neo-Ehrlicha mikurensis, Bartonella Quintana, Bartonella Henslae, *Rickketsia* sp. and *Babesia* sp. Ticks where pooled per dogs. In a total of 350 pooled where analysed by DNA extraction followed by the PCR amplification. MATERIAL AND METHODS Qiagen kit for tissue and cysts is used to extract the DNA from ticks. The DNA tested for *Babesia* sp. by conventional PCR. Borrelia burgdorferi is analysed by nested PCR with outer and inner primers. A Touch Downe PCR is used for amplification of Bartonella, Franciella, Neo-Ehrlichia and Rickkettsia. Positive PCR amplification where followed by direct sanger sequencing for species identification. RESULTS AND CONCLUSIONS This study present the presence of Borrelia burgorferi, Neo-Ehrlicha mikurensis, Bartonella Quintana, Bartonella Henslae, *Rickketsia* sp. and *Babesia* sp. in Danish ticks. 17.14% of the ticks are infected with *Rickkettsia* sp.,15% carried *Borrelia burgdorferi*, 7% infected with Babesia, (6% *Babesia canis* and 1% Babesia EU1). 1.14% infected with Neo-Ehrlichia mikurensis, 0.56% *Bartonella henslae*, and 0.3% *Bartonella Quintana*. No detection of Fanciella tularensis is observed during this study. The results verified the existing of several tick-born pathogens in Danish ticks and the prevalence of thus humane pathogens is similar to what are found in Germany, Bellarus and other Europaen countries. But described for the first time in Denmark.

6.3 Parasitic zoonoses/diseases in the Scandinavian-Baltic region

6.3.1 *Echinococcus multilocularis*

P.6.3.1.001 (A)

Northern Slovakia – highly endemic area of alveolar echinococcosis

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In Slovakia, Echinococcus multilocularis, causative agent of human alveolar echinococcosis, was detected in red foxes in 1999 for the first time. Following long-term surveillance of tapeworm occurrence in red foxes revealed the existence of highly endemic areas in northern parts of the country, with overall prevalence rate of 41.6%. In some localities of Presov and Zilina regions the prevalence reaching as much as 60% was recorded. Between 2000 and 2012 in total 24 human cases of alveolar echinococcosis was detected and only three of them did not came from endemic localities of northern Slovakia. Remarkable is the occurrence of the disease in eight persons less than 35 years, including three patients younger than 20 years. Occurrence of *E. multilocularis* in red foxes in whole territory of the country, together with increasing incidence of alveolar echinococcosis in humans and especially in young people indicates the high infectious pressure of northern Slovakia environment and the threat for both, humans and animals. The research has drawn attention to necessity of targeted surveillance and engagement of specialist, veterinarians, zoologists and ecologists, for effective managements of the diseases for effective managements of the diseases prevention.

The work was realized within the frame of the project funded by the Science Grant Agency VEGA 2/0127/13 ERDF project 'Application Centre for Protection of Humans, Animals and Plants against Parasites'(code ITMS: 26220220018)(0.2).

P.6.3.1.002 (A)

Novel screening method for the epidemiological diagnosis of cystic echinococcosis in livestock

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INTRODUCTION Hydatidosis or cystic echinococcosis (CE) causes severe economic loss to livestock. The objective of this study was to assess the antigenic ability of the ocular instillation of a crude fertile hydatid cyst fluid antigen (FHCF-Ag) from

different animal sources, as a screening tool for diagnosis of animal CE.
Material and Methods he antigens were prepared from three different animal sources, camels (crude CHF-Ag), sheep (crude SHF-Ag) and buffalos (crude BHF-Ag) with a total protein content of 100 μg/ml. A total of 397 different animal species were divided into three sub-groups according to the type of the antigen used. The ocular instillation test was performed before animals' slaughter. The antigen was instilled into one eye and after 20 min the test was read and interpreted. Post mortem examination was done to assess the general conditions of the carcasses, the location of the hydatid cysts and confirmation of the diagnosis was made by parasitological and histological examinations.
Results The results showed that the highest percentage of CE infection was recorded in camels (27.1%) followed by sheep (2%). No CE infection was detected among examined goats, cows and buffalos. The higher percentage of infection was observed among older age groups and most of hydatid cysts were located in the lungs, single and not calcified. Crude CHF-Ag was the more sensitive to diagnose CE infection with sensitivity of (61.9%), specificity of (97.4%), PPV of (65%), NPV of (97.1%) with over all diagnostic efficiency of 94.9%).
Coclusions The use of crude CHF-Ag seems to have reasonable antigenic properties, increases the accuracy of the performance of a rapid diagnostic approach and a cost-effective for increasing the quality of data on CE infection among examined animals and could be employed for epidemiological surveillance for CE.

P.6.3.1.003 (A)

Echinococcus multilocularis in rodents (The Emiro Project): the role of rodents for the spread of _Echinococcus multilocularis_ in Sweden

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Echinococcus multilocularis is a parasite of important zoonotic concern in Europe and was first identified in Sweden in a fox shot December 2010. The EMIRO project is a multinational project focused on the rodents acting as an intermediate host in the *Echinococcus* life cycle. The project includes both field (Sweden, Switzerland, and Lithuania) and laboratory (Denmark) investigations. In Sweden, *Arvicolinae* rodents and fox feces/scat are investigated in two low endemic areas of the parasite. Collection methods will provide density estimates of both rodent and fox populations. Collected rodents will be dissected and the livers visually examined for helminths. Samples from suspect liver lesions and parasite eggs isolated from fox feces will be tested for *Echinococcus* DNA using standardized PCR protocols. Feces will also be analyzed to determine fox diet. Fieldwork will occur in spring and fall with the first collection completed Spring 2013. Experimental investigations will be conducted to determine the susceptibility and stages of parasite development in each rodent species tested. Experiments are currently underway in Denmark using the field vole (*Microtus agrestis*). Ideally, future experiments will be planned involving the most abundant species native for Sweden (i.e. *Myodes glareolus* and *Arvicola amphibius*). At the conclusion of the EMIRO project, results from the four partner countries will be combined in the hope of better understanding of the ecology of the target rodent species, their susceptibility to the parasite, and their relationship to the fox. From these observations, we may be better able to predict the risks for further expansion of *Echinococcus multilocularis*. This is particularly important for Sweden where it is still unknown if the cycle has fully established itself and will remain for the future.

6.3.2 Fish-borne parasitic zoonoses in Europe

P.6.3.2.001 (A)

Occurrence and distribution of anisakid nematodes in cod (_Gadus morhua_ L.) from the North Atlantic

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The occurrence and distribution of anisakid nematode in cod from the six following localities were investigated: the north-east Atlantic (the Barents Sea), the west Atlantic (the Irminger Sea), the north-west coast of Greenland, the Sund, the Pomeranian Bay and the Gulf of Gdañsk. In the studied fish, three anisakid species were recorded, i.e. Hysterothylacium aduncum (1171 individuals, 164 cods infected), Anisakis simplex (3153 individuals, 136 cods were infected) and Contracecum osculatum (446 individuals, 90 cods infected) in both the viscera and muscles of the fish. Almost all of the investigated cods were infected with larvae or adult nematode. The highest prevalence was observed for *A. simplex* (100%), *H. aduncum* (95.0%) and *C. osculatum* (67.5%) from the Irminger Sea. The lowest prevalence of all nematodes species was found in the cod from the Pomeranian Bay and the Gulf of Gdañsk. The highest positive correlation was reported between liver weight and the number of *A. simplex* on / in the liver ($R = 0.64778$). In summary, cod from the North Atlantic can be considered as heavily infected with nematodes. Especially the high abundance of *A. simplex* and *C. osculatum* larvae in the muscles and liver is of concern regarding the possible utilization of fresh cod as a food resource. The analysis of the similarity of the cod parasites from the fishing grounds samples showed that there were two basic groups, which tentatively corresponded with two taxonomic division into two subspecies: *G. morhua morhua* (North Atlantic and the Sund) and *G. morhua callarias* (central and eastern basins of the Baltic Sea).

6.3.3 Intestinal protists – diagnostic tools and emerging trends

P.6.3.3.001 (A)

Screening of faecal DNAs for _Blastocystis_ by a novel real-time PCR assay

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Brief Introduction Blastocystis is a common single-celled intestinal parasite found in both healthy individuals and patients with intestinal symptoms, including functional intestinal diseases such as IBS. The genus comprises at least 17 subtypes found in human, other mammalian, and avian hosts. Geographical differences in the distribution of subtypes in humans are seen, and in Europe ST4 appears to be more common in patients with intestinal symptoms than in healthy individuals. Detection of

Blastocystis in the clinical microbiology laboratory has traditionally relied on parasitological methods such as microscopy of faecal concentrates or fixed faecal smears. However, recent development of novel real-time PCR-based assays has enabled more sensitive and accurate detection of Blastocystis with the potential for subsequent subtyping (1, 2). In this study we screened faecal DNAs from patients suspected of parasitic infection for Blastocystis by a novel real-time PCR assay (1).
MATERIALS AND METHODS DNA was extracted from faecal samples using the NucliSENS easyMag protocol (3). A total of 751 faecal DNAs were screened (1), and positive samples were subtyped by barcoding (4, 5). All DNAs had been screened for Dientamoeba fragilis by real-time PCR (6) prior to the study. The Chi Square Test was used to test for association between Blastocystis and *D. fragilis* carriage.
RESULTS AND CONCLUSIONS This is the first time a real-time PCR assay for Blastocystis has been used to screen a larger panel of faecal DNAs. A total of 141/751 (18.8%) were positive; no differences in prevalence were seen among genders or age groups. The association between Blastocystis and *D. fragilis* carriage was highly significant ($P < 0.0005$) adding support to the theory of a common source/exposure and/or that particular intestinal bacterial communities or eco-systems select for the establishment of micro-eukaryote colonisation. The study is ongoing and results will also include data on subtypes.

P.6.3.3.002 (A)
Epidemiology of *Blastocystis hominis* in Indonesian immigrant workers

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BACKGROUND Blastocystis hominis is one of the most common intestinal protozoans in humans. Studies showed that developing countries have higher prevalence of Blastocystis hominis infection. The purpose of this study was to determine the molecular epidemiology of Blastocystis hominis in Indonesian immigrant workers in Taiwan.
METHODS Fresh fecal samples were collected, precipitated and examined by microscope for parasites studyies. After DNA extracted from stool samples in which positive for Blastocystis hominis infection, ssu rDNAs genotyping was performed by Nested polymerase chain reaction and phylogenetic analysis.
RESULTS Thirty of the total 128 examined immigrants (21.8%) are positive for intestinal parasitic infections and 28 patients are Blastocystis hominis infection. Twenty-five of the 28 (89.2%, 25/28) samples are positive in PCR study. In genotypic analysis, the most common genotypic is subtype 3 (52.0%, 13/25), followed by subtype 2 (36.0%, 9/25) and subtype 1 (12.0%, 3/25). Phylogenetic analysis suggested the strains isolated form human are similar to isolated from different hosts.
CONCLUSION Blastocystis hominis is a zoonotic infection, which is transmitted by the fecal-oral route. The studies indicated that Blastocystis hominis in humans were closely related to animals. In the future, more public education and proper management may help to reduce Blastocystis hominis transmission contamination.

6.3.4 Other zoonotic parasites in man and wildlife

P.6.3.4.001 (A)
Warble infestations from *Hypoderma tarandi* (Diptera; Oestridae) recorded for the first time in West Greenland muskoxen

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INTRODUCTION In the northern hemisphere, Caribou (*Rangifer* spp.) populations are known to be infested with the skin-penetrating ectoparasite, Hypoderma tarandi (Diptera; Oestridae). Although regarded as host specific, *H. tarandi* have been reported from other species, and has become of increasing concern as a zoonosis infecting humans.
MATERIAL AND METHODS In February 2012, in Kangerlussuaq, West Greenland, concurrent with the hunting of muskoxen, we examined the carcasses for muscle and tissue parasites, and recorded warble larvae infestations.
RESULTS DNA extracted from samples of larvae was amplified targeting 579 bp of the COI gene, and subsequently sequenced, to be confirmed as *H. tarandi*.
CONCLUSION Infestation by Oestrid flies has not previously been reported in muskoxen in West Greenland. Hypoderma tarandi is normally regarded as host specific and infestations found in muskoxen seem to be the result of a cross-contamination from caribou, in which infestations are highly prevalent. Cross transmission studies on *H. lineatum* indicate the possibility of multiple-species infections but with limited development beyond first-instar larvae. This study shows development beyond this larval stage in muskoxen. Whether development to subsequent larval instars or into adult flies is possible regarding *H. tarandi* with muskoxen as hosts is not known. There are no records of complete development of *H. tarandi* in humans. However, the reported cases of ophthalmomyiasis caused by *H. tarandi*, which often lead to loss of sight, are of concern. Such cases are uncommon, but have been reported from the Canadian Arctic, Scandinavia and the Netherlands. These reports have led to an increased awareness of *H. tarandi* as a zoonosis.

P.6.3.4.002 (A)
Naturally acquired fatal toxoplasmosis in a great spotted woodpecker

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Toxoplasma gondii is a widespread zoonotic parasite of warm-blooded animals, including birds. There is only one previous report of naturally acquired clinical toxoplasmosis in a woodpecker – here we describe a case of fatal toxoplasmosis in a great spotted woodpecker (Dendrocopos major) from Norway. This juvenile male great spotted woodpecker was found dead and submitted for post-mortem examination, which included routine necropsy, histopathologic evaluation, and selected further investigations. The gross findings were unspecific: consolidated, edematous lungs and splenomegaly. The histopathological diagnoses included acute nec-

rotizing interstitial pneumonia, splenitis, and hepatitis. The protozoan parasites detected were confirmed as *T. gondii* with immunohistochemistry, and were present in all the organs examined. Toxoplasmosis was acute and generalized, and the cause of death of this woodpecker. Direct genetic characterization of the causative *T. gondii* strain was based on an analysis of seven microsatellite markers (B18, TUB2, TgM-A, W35, B17, M33, and M48), and revealed that the woodpecker had been killed by a *T. gondii* strain that belongs to genotype II. This is surprising because genotype II strains typically cause subclinical infections. Genetic characterization of the parasite strains that cause severe toxoplasmosis in different host species increases our understanding of the effect the genotype of the parasite has on the outcome of the infection. Little is known of this effect in most animal hosts, and thus even a single case report adding to this information is valuable.

P.6.3.4.003 (A)

Alaria alata in mammals of Latvia

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Intestinal parasite Alaria alata is a digenea trematode which is widespread throughout all the Europe. In the life cycle of *A. alata* is includes two intermediate hosts (snails and amphibians) and definitive host (carnivores – raccoon dogs and red foxes). However the life cycle can be extended by paratenic hosts as wild boars and other mammals. Recent findings of mesocercariae of *A. alata* in wild boars indicate that they could potentially infect humans. For the last few years the numbers of raccoon dogs and wild boars are increasing. In 2010 the population of raccoon dogs was estimated with 26934 individuals and 29152 individuals in 2012 but wild boars increased from 67150 to 72508 specimens in Latvia. On the contrary the number of red foxes is decreasing from 34039 to 32805 individuals (State Forest Service, unpublished data). Overall 321 red fox and 206 raccoon dog small intestines were examined using sedimentation and counting technique during 2010 to 2012. Also muscle samples of 4088 wild boars were examined during the official Trichinella inspection with artificial digestion and magnetic stirrer method analyzing 50 g of diaphragm muscle sample. The prevalence of *A. alata* in definitive hosts – red foxes and raccoon dogs – was 82% and 86%, while in paratenic hosts as wild boars – 7%. In general mean intensity of *A. alata* was higher in raccoon dogs (420); it varies from 262 to 473 in different regions of Latvia. Mean intensity in red foxes is 138, but varies from 91 to 161. The mean intensity in wild boars reached 3 mesocercariae per sample in Latvia. Alaria alata were found in considerably high prevalence in all regions and animals from examined species in Latvia. High numbers of intensity and prevalence are caused by rather high animal population density in Latvia.

P.6.3.4.004 (A)

Toxoplasma gondii antibody prevalence in Estonian wild boars

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Annually, 20 000 wild boars (Sus scrofa) are being hunted for human consumption in Estonia. Meat of animals carrying *Toxoplasma gondii* may be a source of human *T. gondii* infections, if eaten insufficiently cooked. This study aimed to estimate *T. gondii* antibody prevalence in the wild boar population of Estonia: the proportion of animals that had encountered the parasite and produced specific IgG antibodies against it. During the hunting season from 1st of October 2012 to 28th of February 2013, voluntary hunters sampled hearts of wild boar. Meat juice from thawed samples were diluted 1:40 and screened with a commercial direct agglutination test. Chi-square analysis was used to evaluate differences between the categorical variables: gender, estimated age (≤1 or >1 year of age), and county where the animal was shot. Out of 471 individual samples submitted, 113 (24.0%, 95% CI 20.3–28.1) were defined antibody positive. This result is similar to recent findings in Finland and Latvia and show the wild boars in Estonia are commonly exposed to this important zoonotic parasite. No differences in antibody positivity were found between genders ($P = 0.92$, $X^2 = 0.01$), age groups ($P = 0.68$, $X^2 = 0.17$), or the counties ($P = 0.16$, $X^2 = 17.93$).

P.6.3.4.005 (A)

Trichinella sp. in pine martin (*Martes martes*) and stone martin (*Martes foina*) in Latvia

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Pine martin and stone martin populations of Latvia territory are growing because of animal good adaptation to urban territories and high number of glades witch increases supplies of feed. Knowledge in wildlife distribution of zoonotic agent Trichinella is important because wild animals represent the most important source of infection for domestic pigs, which in turn are the main source of infection for humans. Till now data about *Trichinella* spp. invasion in pine and stone martin in Latvia are fragmentary. Seven stone and 41 pine martin muscle samples were collected in hunting seasons of winter in 2012/2013 from different territories of Latvia using hunting method of frame-like traps. All samples for *Trichinella* spp. larvae detection was analyzed in accordance with the requirements in Annex I of the Commission Regulation (2005). Meat sample of at least 25 g material consists of muscle tissue taken from foreleg tissues. All meat samples were stored frozen at −20°C until analyzes. *Trichinella* spp. species identification was done by multiplex PCR methods. Total *Trichinella* spp. prevalence in *Martes* sp. was 47.9%. That is similar to prevalence detected for raccoon dog in Latvia. Prevalence in stone martin was 57.1% and pine martin was 46.1%. Larvae number per one gram musculature vary from 0.04 to 25. Trichinella britovi is the most widespread etiological agent of Trichinella infection in wild and domestic animals and has the widest geographical range, occurring wildlife of the temperate areas of the Europe and Asia. This is also the most common agents of Trichinella infection in Latvia and it can be transmitted from animal to animal through consumption of scraps from sylvatic carnivores and wild boars. The preliminary results of study show that in Latvia zoonotic agent Trichinella is circulating in wildlife and Martes species are frequent natural reservoirs for this parasite.

6.4 Epidemiology, case studies, surveillance & information to the public

P.6.4.001 (A)

Endoparasites of buzzards (*Buteo* spp.) in Slovakia

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Three raptor species from genus Buteo occur in Slovakia – Common-Buzzard (Buteo buteo), Rough-legged Buzzard (Buteo lagopus) and Long-legged Buzzard (Buteo rufinus). The Common-Buzzard is the most common raptor not only in Slovakia but also in Europe. The main diet component of these birds represents small rodents, especially *Microtus arvalis*. Our pilot study was focused on detection of endoparasites in Buzzards from Slovakia. We examined 53 Common-Buzzard, 1 Rough-legged Buzzard and 1 Long-legged Buzzard. The raptors were subjected to parasitological dissection, the samples of pectoral muscles were examined for trichinellosis using artificial digestion method and the fecal samples were examined using standard flotation technique for the presence of parasite eggs and coccidian oocysts. Parasitological dissection revealed 30% of birds being infected with parasites from the class Trematoda, in 40% were determined specimen from class Cestoda, in 6% of individuals Acanthocephalan parasites were found and the most common was Nematode infection (64%). Coprological examination detected 42.4% prevalence of *Capillaria* spp., in one Long-legged Buzzard eggs of the genus Cyathostoma were found and in 15.2% of samples intestinal coccidian oocysts were present. Although the raptors are potential hosts of *Trichinella pseudospiralis* parasite, no positive specimen was found within our study. Our results indicate that parasite fauna of raptors from the genus Buteo is quite diverse. In the following research we focus on parasite species identification and detection of blood parasites by means of molecular methods.

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P.6.4.002 (A)

Socioepidemiological study of farmers' behaviour in preventing parasitic infections of calves in Estonia

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Estonian cattle farms vary in herd size from a few animals to 2100 dairy cows and also housing conditions and management differ. The major factor is people and their beliefs of what strategies work in running the farm. Logically, beliefs affect the behaviour, behaviour affects the management, management affects the level of hygiene, and hygiene has direct influence on the successful control of many parasitic diseases. Parasitic diseases are particularly important to young animals. This study investigates farmers' beliefs regarding conditions that affect calves, using a questionnaire designed according to the Theory of Planned Behaviour (TPB). The method enables evaluation of farmers' actual behaviour through behavioural intention analysis. The target population was Estonian dairy farms with a herd size of at least five dairy cattle. Our selection criteria were met by 1688 herds registered in the Estonian Agricultural Registers and Information Board. We sent out 1468 questionnaires by mail in April 2013. The announced deadline for the completed questionnaires is 7th May 2013. The response rate is already exceeding 33% (1st May 2013). Preliminary evaluation shows that the average farmer is 52 years old and farmers of all age groups seem to value veterinarians and seminars as most important information sources regarding calf management.

P.6.4.003 (A)

Coccidiosis in Finnish dairy calves and association with acute phase response

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The study aimed to investigate the occurrence of *Eimeria* spp. in Finnish dairy calves, and to examine associations between acute phase proteins (APP) and coccidiosis. On farm visits (n = 22) all the calves ≤2 months of age (younger calves; n = 142) were clinically examined and blood and faecal samples were obtained. Additional samples were collected from randomly selected calves aged 2–6 months (older calves; n = 154). Blood samples were analysed for APPs (haptoglobin; Hp and serum amyloid A; SAA) and faecal sample for intestinal pathogens (*Eimeria* spp., rotavirus, bovine coronavirus, *Cryptosporidium* spp., *Giardia* spp.). *Eimeria* spp. was found in 30.8% and in 65.6% of younger and older calves, respectively. Eimeria bovis or *E. zuernii* were detected in 23.2% and 26.1% of samples, respectively. In younger calves suffering from diarrhea, *E. bovis* or *E. zuernii* were more often found as mono-infections compared to other intestinal pathogens. No intestinal pathogens were found in 25.4% of younger calves. Mixed linear regression models were used to investigate the association between APP concentration and coccidiosis in younger calves. Coccidiosis was classified mild (oocyst count per gram faeces (opg) 1–500 opg + diarrhea - pathogenic species or opg 1–500 opg - diarrhea ± pathogenic species or opg >500 – pathogenic species – diarrhea) or severe (opg >500 opg + diarrhea ± pathogenic species or opg >500 opg - diarrhea + pathogenic species or opg 1-500 + diarrhea+pathogenic species). Farm was included as random factor and other clinical diseases were controlled. Decreased Hp concentration was associated with mild clinical coccidiosis (P = 0.02) with a tendency to decrease with severe forms of clinical coccidiosis (P = 0.12). No statistical associations were found between coccidiosis and SAA concentrations. In conclusion, pathogenic Eimeria species are common intestinal pathogens in Finnish dairy calves and Hp is negatively associated with severity of clinical coccidiosis.

P.6.4.004 (A)

Prevalence of intestinal parasitic infections among food handlers in Sari, Northern Iran

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The purpose of this study was to determine the prevalence of carriers of intestinal parasites among food handlers attending the public health center laboratory in Sari, northern Iran for annual check-up. The study was conducted from August 2011 to February 2012. Stool samples were collected from 1041 male and female food handlers of different Jobs aged between 18 and

63 years and were examined following standard procedures. Sociodemographic, environmental and behavioral data of the food handlers were recorded in a separate questionnaire. Intestinal parasites were found in 161(15.5%) of the studied samples. Seven species of protozoan or helminth infections were detected. Majority of the participants were infected by Giardia lamblia (53.9%) followed by *Blastocystis hominis* (18%), *Entamoeba coli* (15.5%), *Entamoeba histolytica/dispar* (5.5%), *Cryptosporidium* Sp.(3.1%), *Iodamoeba butschlii* (3.1%) and *Hymenolepis nana* (1.9%) as an only helminth infection. The finding stressed that food handlers with different pathogenic organisms may pose significant risk on the consumers. Routine screening and treatment of food handlers is a valuable tool for prevention of foodborne infections.

6.5 Parasites – treatment and control

P.6.5.001 (A)

Evaluation of soil microfungi as biological control agents against ascarid eggs

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Thick-shelled ascarid eggs have been reported to remain infective in the environment for several years, thus posing a prolonged risk of infection to animals and/or humans. The following *in vitro* study was therefore conducted to evaluate the negative impact of two species of naturally occuring soil microfungi (*Pochonia chlamydosporia* and *Paecilomyces lilacinus*) on the viability of *Ascaridia galli*, *Toxocara canis* and *Ascaris suum* eggs. Approximately 150 fresh eggs of each ascarid species were embryonated on a 2% water agar in Petri dishes with or without a fungus (*P. chlamydosporia* or *P. lilacinus*). On days 7, 14, 21, 28, 35 and 42 post experimental set up (p.s.), the viability of the eggs was evaluated (destructive sampling). By day 14 p.s., *P. chlamydosporia* had reduced the viability of *A. galli* and *T. canis* eggs by 70–86% and 52–67%, respectively, compared to the controls. In contrast, *P. lilacinus* had reduced the viability of *A. galli* and *T. canis* eggs by only 17–30% and 6–28%, respectively. Neither fungal species was found to be effective against *A. suum* eggs (<4% reduction in both cases). These results indicate interspecies differences in the susceptibility of ascarid eggs to microfungi. For both fungi, enzymatic degradation of egg shell protein and chitin seemed to be the primary mechanism in degenerating the *A. galli* and *T. canis* eggs. The present study has demonstrated that *P. chlamydosporia* may potentially be utilized as a biological control agent against *A. galli* and *T. canis* eggs in the environment.

P.6.5.002 (A)

The effects of *Moringa oleifera* seed powder on turbidity and sedimentation of *Cryptosporidium* spp. in wastewater

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The use of different types of wastewater for irrigation in agriculture is common practise in many countries due to limited freshwater resources. Contamination of fruits and vegetables with pathogenic microorganisms, including *Cryptosporidium* spp., may occur when the irrigation water or water used for postharvest washing of the produce is contaminated. A laboratory study was carried out to investigate the effect of a coagulant from the seeds of Moringa oleifera (MO) in reducing Cryptosporidium parvum oocysts and turbidity in Danish wastewater. To each of five time points, 12 replicates of 500 ml wastewater samples were added 1.2×10^5 oocysts per litre. Half of the replicates were added 4 ml/l of a 5% w/v MO extract, while the other half was left untreated. The samples were stirred slowly for 20 min and subsequently left to sediment for 15, 30, 45, 60 or 90 min. In wastewater from a Danish treatment plant MO extracts were effective in reducing the number of Cryptosporidium oocysts by $93 \pm 3.2\%$ after 90 min of sedimentation and the turbidity to 9.8 ± 1.8 NTU, corresponding to a turbidity removal of 95%. MO is readily available in many tropical countries and can be used by farmers for water treatment. Yet, in this study, a large number of oocysts remained in the wastewater after treatment. This was likely because the proportion of oocysts and active component from MO extract was imbalanced, with more oocysts added than the MO extract could adhere to. Additional experiments with water samples containing fewer and/ or naturally occurring Cryptosporidium oocysts are needed to establish whether MO extract can be used to effectively treat and obtain safe water free of Cryptosporidium oocysts.

6.6 Molecular parasitology and evolution

P.6.6.001 (A)

Challenges and directions for developments of barcoding of haemosporidian (Haemosporida) parasites

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DNA barcoding has become popular and important tool in the identification of a variety of animals, particularly parasites. Different PCR-based methods have been developed for detection and identification of haemosporidian (Haemosporida) parasite species. As a result, numerous nucleotide sequences (lineages) of the parasites and data about their host range and distribution have been accumulated in GenBank and MalAvi database. On the other hand, the traditional systematics and taxonomy of haemosporidian parasites are based mainly on morphological characters visible under microscope during development in blood of vertebrate hosts. Recently, numerous studies developed molecular characterization (barcodes) for avian blood parasites from the genera Haemoproteus, Plasmodium and Leucocytozoon, but the predominant number of genetic sequences have been identified only to generic level. When applying traditional microscopic examination together with molecular identification in order to develop barcodes for bird haemosporidians, several difficulties usually occur: (i) poor quality of the blood smears (shortcomings of their preparation, fixation or staining), (ii) insufficient experience in the taxonomy and systematics, (iii) light intensity of parasitemia and/or absence of all blood stages necessary for morphological identification, and (iv) co-infections which are common in wild birds. Unfortunately, an increasing number of deposited sequences are also accompanied with incorrectly identified parasites; that is an obstacle for developing barcoding. In order to avoid misidentification it is recommended (i) to use spe-

cies specific primers or single infections only; (ii) to use experimental infections; (iii) to deposit voucher specimens of parasites in prominent museum collections; (iv) to use blood samples from the type hosts, especially for specific parasites; and (v) to apply modern phylogenetic approach and expertise of taxonomists.
This study was supported by European Union Structural Funds project 'Postdoctoral Fellowship Implementation in Lithuania' (VP-3.1-SMM-01-V-02-004).

P.6.6.002 (A)

A comparative transcriptomic and proteomic investigation of host cells response during *Toxoplasma gondii* and *Neospora caninum* invasion of human astrocytes

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Toxoplasma gondii and *Neospora caninum* are intracellular protozoan parasites from the phylum Apicomplexa. Both parasites share many morphological and genetic features, but have diverse host preferences. While *T. gondii* can infect any warm-blooded animal including humans, *N. caninum* does not infect humans and is widely spread through vertical transmission in cattle. In immunosuppressed individuals, the chronic stage of *T. gondii* can cause Toxoplasma encephalitis, whereas neosporosis results in neuromuscular disease in dogs and abortion in cattle. The basis of host preference in these two parasites is unknown, but could be due to differences in tropism to specific host cells. To test this hypothesis, we investigated the differential expression of host-cell and parasite genes/proteins during the invasion of human astrocyte (HA) cells by both Toxoplasma and Neospora. Parasites and HAs were cultured *in vitro*; cells were infected with type III *T. gondii* VEG strain and *N. caninum* Liverpool isolate strain. Samples were collected at 0, 4 and 16 h post infection for both transcriptomic and label-free proteomic analysis. A number of differences in host cell responses were noted between Toxoplasma and Neospora infection. Among these were differences in expression related to the type1 interferon pathway in *N. caninum* infected cells. In addition, the transcriptomics data from *T. gondii* suggests the up-regulation of various suppressive factors that prevent activation of the host response. These data suggest that the two parasites stimulate some fundamentally different host cell responses, with Nesopora, surprisingly, acting more like a 'virus infection' than an obligate intracellular protozoan parasite.

6.7 Parasite ecology

P.6.7.001 (A)

Studies of Rhipidocotyle fennica Gibson, Taskinen & Valtonen 1992 (Digenea: Bucephalidae), parasitising bivalvia *Anodonta anatina*, based on DNA sequences

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The history of investigations of European freshwater bucephalid species is complicated. The life cycle of bucephalids involves a distinctive furcocercous cercaria that emerges from bivalves and encysts in the second intermediate host, the fish, which in turn is eaten by definitive host (Baturo, 1977). Gibson, Taskinen & Valtonen (1992) showed the presence of a new bucephalid species, Rhipidocotyle fennica in central Finland in addition to two bucephalids, *R. campanula* and Bucephalus polymorphus, already known in European freshwater habitats. The cercaria of *R. fennica* with long filamentous furcae closely resembles cercaria of *B. polymorphus* (Taskinen et al. 1991), while cercaria of *R. campanula* clearly differs. Larval bucephalids found in unionids have until recently been called *B. polymorphus* in some publications (Yanovich, Stadnichenko, 1997). For this study parthenitae of *Rhipidocotyle* spp. infecting Anodonta anatina were gathered from freshwater bodies in Finland, Lithuania and Ukraine. Comparative analysis using ribosomal ITS2 and 28S DNA sequences was performed. On the basis of molecular data, we recognised, that bucephalid cercariae with long filamentous furcae parasitising *A. anatina* belongs to species *R. fennica* in all studied populations. So, this species is not endemic for Finland. All previous records of *B. polymorphus* in the unionid clams must be attributed to *R. fennica*. Bucephalus polymorphus is specific to *Dreissena* spp. (Baturo, 1977) and reports of its presence in unionids are doubtful.
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P.6.7.002 (A)

DNA based analysis of the life cycles of *Phyllodistomum* spp. (Digenea, Gorgoderidae): current status of knowledge and perspectives

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At present, around 14 species of Phyllodistomum have been recorded in freshwater fishes in Europe. Only few life cycles were determinate by experimental infections, but these results are controversial. The most common type of life-cycle described is that characterized by cystocercous cercariae, but rhopalocercous and a microcercous cercariae are also known. Recently used molecular techniques have a great potential as an alternative to the classical approaches for the elucidation of digenean life cycles. Material was collected in different water bodies of Europe. ITS2 and 28S DNA sequences of adult and larval stages of *Phyllodistomum* spp. were obtained and compared. Life-cycle described for the type-species *P. folium* (Olfers, 1816) by Sinitsin (1905) can apparently be discounted. Adult specimens, obtained from type host, pike, and microcercous cercariae developing in Dreissena polymorpha, showed no match between the DNA sequences. The match was detected between sequences of adult *P. folium* and cystocercous cercariae from sphaeriid bivalves. Cystocercous cercariae from *Pisidium amnicum*, known as *P. elongatum*, as well as adult *P. elongatum* Nybelin, 1926 from roach, showed no differences from *P. folium*. Consequently, the validity of *P. elongatum* is doubtful. Rhopalocercous cercariae, Cercaria duplicata von Baer, 1827, from *Anodonta anatina* have been linked to *P. elongatum* by Orecchia et al. (1975) and *P. angulatum* von Linstow, 1907 by Ivantsiv and Kurandina (1985). The results of our molecular study do not justify any of these experimental findings. This study supports the existence of a pressing need for all aspects of biology and taxonomy of European phyllodistomes to be thoroughly reinvestigated.
This research was funded by a grant (No. MIP-52/2013) from the Research Council of Lithuania.

P.6.7.003 (A)

First description of cercaria of *Stephanoprora pseudoechinata* (Olsson, 1876) (Digenea: Echinostomatidae) using morphological and molecular data

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The trematode *Stephanoprora pseudoechinata* is an intestinal parasite of various fish-eating birds. Metacercariae were reported from fishes, Alburnus alburnus, Gasterosteus aculeatus, Pungitius pungitius in the Gulf of Riga (Krasnolobova, 1971). The first intermediate host of this parasite was unknown. In August 2011 a preliminary survey of trematodes of birds in the Yagorlytska Bay of the Black Sea (Kherson region, Ukraine) was made. Adults of *S. pseudoechinata* were recovered from the intestine of *Larus melanocephalus*. During the helminthological studies of the brackish water gastropods Hydrobia acuta in the Yagorlytska Bay in 2012 we have found large-tailed cercariae. One of 1817 examined molluscs was infected. Morphological examination was conducted on living and fixed specimens. Morphological features observed led us to conclude that this species was an echinostomatid fluke, and it was identified as *Stephanoprora* sp. We used nuclear ribosomal (28S and ITS2) DNA sequences to establish the link between the cercaria infecting *H. acuta* and the trematodes previously collected from *L. melanocephalus*. The results demonstrated that the DNA sequences from the cercaria and adult of *S. pseudoechinata* are identical. The life cycle of the *S. pseudoechinata* species is similar to that of *S. denticulata* from Denmark (Køie, 1986) and *S. uruguayense* from Argentina (Ostrowski de Núñez, 2007) in that hidrobiid molluscs and fishes act as intermediate hosts. Morphology and morphometry of *S. pseudoechinata* cercaria is most similar to that of *S. denticulata* from Europe. Cercariae of *S. pseudoechinata* can be definitely identified to species level only using molecular markers.

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P.6.7.004 (A)

Complete synchronous sporogony of the malaria parasite *Plasmodium relictum* (lineages pSGS1 and pGRW11) in mosquitoes of *Culex pipiens* group

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Plasmodium relictum is a widespread invasive agent of avian malaria, responsible for acute, chronic and debilitating diseases in many species of birds. Recent PCR-based studies revealed astonishing genetic diversity of avian malaria parasites (genus *Plasmodium*), with numerous genetic lineages deposited in GenBank. Many studies addressed distribution and evolutionary relationships of avian *Plasmodium* spp. lineages, but information about patterns of development of different lineages in vectors remains insufficient. We investigated sporogonic development of two widespread mitochondrial cytochrome b lineages (cyt b) of *P. relictum* (pSGS1 and pGRW11) in mosquitoes of the *Culex pipiens* group (*C. pipiens pipiens* and *C. pipiens f. molestus*). Genetic distance between these lineages of malaria parasites is 0.2%; they fall in a well-supported clade in the phylogenetic tree. These parasites were isolated from 1 common crossbill (Loxia curvirostra, lineage pSGS1) and 1 house sparrow (Passer domesticus, pGRW11); the strains were multiplied in domestic canaries who served as donors of malarial gametocytes to infect mosquitoes. Mosquitoes were allowed to take infected blood meal and then dissected on intervals to study development of sporogonic stages. Both lineages developed synchronously and completed sporogony in both forms of *C. pipiens* mosquitoes, with infective sporozoites reported in the salivary glands on the days 14–15 after infection. Ookinetes, oocysts and sporozoites of all strains were indistinguishable morphologically. This study shows that (i) the patterns of sporogonic development of the closely related lineages pSGS1 and pGRW11 of *P. relictum* are similar, and (ii) the phylogenetic trees based on the cyt b gene likely can be used for predicting sporogonic development of genetically similar avian malaria lineages in mosquito vectors. Our experience also show that *C. pipiens f. molestus* is a particularly convenient experimental vector because it is easy to cultivate in the laboratory conditions.

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6.8 Methods in parasitological research

6.8.1 Geospatial Health: Mapping and modelling parasitic infections

P.6.8.1.001 (A)

Modeling the spatial distribution of trematode vector snails responsible for transmission of schistosomiasis, fascioliasis, and paramphistomosis and the impact of climate changes

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The ecological niche of vector snails, involved in transmission of diseases caused by intestinal worms, are modelled and mapped, and climatic parameters that may serve as predictors are identified and quantified. Once a mathematical relationship between climate predictors and vector has been established, it is possible to predict the potential distribution of the snail (and in turn disease distribution) in areas where no data exists. These modelling results can serve as valuable information when planning health interventions. We use georeferenced vector snail observations from two snail collection surveys in 1988 (364 localities) and 2012 (64 localities) together with climate parameters serving as predictors for the potential niche of vector snails. The snail species involved in the transmission of urinary- and intestinal schistosomiasis (*Bulinus globosus*, *Biomphalaria pfeifferi*, respectively) were collected together with the snail *Lymnaea natalensis,* responsible for the transmission of fascioliasis and paramphistomosis. Satellite observation data on temperature, rainfall, and NDVI (Normalised Difference Vegetation Index) were downloaded on a 10 km resolution from NOAA data servers for 1988 and 2012; formatted in the ArcMap GIS-software to comply with MaxEnt, used for the niche modelling. A model for each of the two periods are being developed and output can be interpreted as distribution change in two climate situations, similar to the effect of climate change. Preliminary statistical analysis of the snail distribution show that the snail dataset is suitable for modeling in MaxEnt and early results show consistency with observed distributions. Climate data are of high quality and performs well as predictors. Conclusions on development during the 24-year period from 1988 to 2012 remain.

This study serve as a baseline for modeling of snail distribution in a future climate and can give us valuable information on

the burden of snail borne diseases in the future, globally and locally.

6.8.2 Diagnostic tools in parasitology

P.6.8.2.001 (A)

The use of pooled sodium acetate acetic acid formalin - preserved faecal specimens for detection of intestinal parasites

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Diagnosis of intestinal parasites is depending on microscopic examination of stool specimens. This study aimed at comparing detection of intestinal parasites from single fresh unpreserved stool sample versus sodium acetate acetic acid formalin (SAF)-preserved pooled samples, and stained with chlorazol black dye in routine practice. Unpreserved samples were collected from 120 patients and represented as Group I. Other three SAF-preserved samples were collected from the same patients over a 6 day period and represented as Group IIa, IIb, IIc. The latter group was equally subdivided into two subgroups. The first subgroup of each of the three samples was examined individually, whereas the second subgroup of each were pooled and examined as a single specimen. All groups were examined by the routine diagnostic techniques, however, in group II when the diagnosis was uncertain, the chlorazol black dye staining procedure was carried out. Results demonstrated that out of 120 patients, 74 only continued the study. Twelve cases (16%) were positive in group I, compared to 29 cases (39%) in the subgroups examined individually, and 27 cases (36%) in the pooled subgroups. Therefore, the use of the multiple samples strongly improved the detection of intestinal parasites. In addition, pooling of preserved fecal samples is an efficient and economical procedure for the detection of parasites. Furthermore, the chlorazol black dye was simple and effective in detection the nuclear details of the different parasites. Thus, we concluded that, this modified technique is recommended to be used in routine clinical practice.

P.6.8.2.002 (A)

Molecular prospecting for *Diplostomum* spp. in the Russian North-West

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Metacercariae of trematodes belonging to the genus Diplostomum parasitize freshwater fishes worldwide, causing sometimes high fish mortality. So identification of *Diplostomum* spp. is important in different studies. In this study, sequences from the ITS1 + 5.8S+ITS2 region of rDNA were used to distinguish larval and adult parasites *Diplostomum* spp. collected from diverse snails, fish and birds from different lakes of the Russian North-West. The findings were corroborated with morphological description of parasites. Morphologically Species were identified based on keys of Niewiadomska (1984, 1989), Niewiadomska, Kieseline (1994), Shigin (1986, 1993), Sudarikov et al. (2001). Amplification and sequencing was performed using the primers and PCR protocols of Moszczynska et al. (2009). Six species were detected (*D. huronense*, *D. paracaudum*, *D. pseudospathaceum*, *D. baeri*, *D. mergi*, *D. spathaceum*). Gulls had the most diverse and abundant parasites invasion. Great Crested Grebe (*Podiceps cristatus*) was infected by *D. mergi* only. Trematoda *D. pseudospathaceum* was recorded in Osprey (*Pandion haliaetus*) parasite fauna for the first time. Using matching of molecular and morphological features of parasites in snails, fish and birds life-cycles of *D. paracaudum*, *D. pseudospathaceum* and *D. baeri* were revealed. Although this is not so high diversity, other species are likely to exist in the study area.

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6.11 Parasite immunology and pathology

P.6.11.001 (A)

Determination of *Toxoplasma gondii* Parasitic Load in Balb/c Mice Immunized with ESA of RH Strain using Real Time Q-PCR

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INTRODUCTION *Toxoplasma gondii* is an obligatory intracellular parasite in different cells of human beings and animals. The aim of this study was to evaluate presence and movement trend of *T. gondii* tachyzoites in different tissues of Balb/c, after immunization with Excretory Secretory Antigens (ESA).

MATERIAL AND METHODS This experimental survey has been performed on 24 Balb/c mice in case and control groups. For immunization of mice, two times, intervals two weeks, case group (n = 12) received 40 μl ESA+40 μl Adjuvant and control group got 40 μl PBS + 40 μl Adjuvant. Two weeks after the second immunization, mice were challenged with 1×10^4 alive and the active tachyzoites of *T. gondii* RH strain and on days 1,2,3 and the last day (before death) after challenge, different tissues (eye, muscle, kidney, heart, brain, spleen, blood and liver) of three mice from each group were prepared and DNA extraction, parasite load of tissues has been evaluated by Real Time Q-PCR.

RESULTS Toxoplasma after intraperitoneal injection, in both case and control groups were able to movement to various tissues. In the case group receiving Excretory Secretory Antigens (ESA), parasite load in eye, kidney, brain, blood and liver was less than control group.

CONCLUSION Hence, ESA reduced the the parasite load, but could not inhibit the distribution and presence of Toxoplasma in different tissues.

KEYWORDS Movement trend, *Toxoplasma gondii*, tissue, Balb/c, immunization, Excretory Secretory Antigens (ESA), Real Time Q-PCR.