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Aetiology, epidemiology and clinical characteristics of acute moderate-to-severe diarrhoea in children under 5 years of age hospitalized in a referral paediatric hospital in Rabat, Morocco

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The objective of the study was to describe the aetiology, epidemiology and clinical characteristics of the principal causes of acute infectious diarrhoea requiring hospitalization among children under 5 years of age in Rabat, Morocco. A prospective study was conducted from March 2011 to March 2012, designed to describe the main pathogens causing diarrhoea in hospitalized children >2 months and less than 5 years of age. Among the 122 children included in the study, enteroaggregative *Escherichia coli* (EAEC) and rotavirus were the main aetiological causes of diarrhoea detected. Twelve (9.8 %) children were referred to an intensive care unit, while two, presenting infection by EAEC, and EAEC plus *Shigella sonnei*, developed a haemolytic uraemic syndrome. Additionally, six (4.9 %) deaths occurred, with EAEC being isolated in four of these cases. Diarrhoeagenic *E. coli* and rotavirus play a significant role as the two main causes of severe diarrhoea, while other pathogens, such as norovirus and parasites, seem to have a minimal contribution. Surveillance and prevention programmes to facilitate early recognition and improved management of potentially life-threatening diarrhoea episodes are needed.

INTRODUCTION

Diarrhoeal disease remains a major contributor to illness and death among children less than 5 years of age in low- and middle-income countries, and is also a relevant cause of morbidity among international travellers to these areas (Liu *et al.*, 2012). Indeed, paediatric diarrhoeal disease still accounts for >800 000 deaths per year globally (approximately 11 % of the 7.6 million estimated annual global child deaths) (Lanata *et al.*, 2013; Liu *et al.*, 2012). Nonetheless, diarrhoea-associated mortality is decreasing globally by about 4 % yearly; however, the decline in incidence is modest (Levine *et al.*, 2012; Liu *et al.*, 2012). It is considered that yearly diarrhoea accounts for approximately 2.5 billion cases in children less than 5 years old (UNICEF/WHO, 2009), affecting up to 60 % of travellers to some low-income areas (Gascón, 2006; Ruiz *et al.*, 2007; UNICEF/WHO, 2009). Additionally, different diarrhoea-related severe sequelae have been described, including Guillain–Barré syndrome, haemolytic uraemic syndrome (HUS) and reactive arthritis (Fischer Walker *et al.*, 2013). In low- and middle-income countries, in which children may have several episodes per year, diarrhoea may lead to nutritional deficits, and subsequent growth stunting and decreased cognitive function (Fischer Walker *et al.*, 2013). Regarding Morocco in 2009, one study (Oudaïna *et al.*, 2009) showed that stature-ponderal delay was related to the presence of diarrhoeagenic parasites in at least one of each six children. These high levels

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Abbreviations: CRP, C-reactive protein; DAEC, diffusely adherent *E. coli*; EAEC, enteroaggregative *E. coli*; EHEC, enterohaemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; HER, Hôpital d'Enfants de Rabat; HUS, haemolytic uraemic syndrome; ICU, intensive care unit; IQR, interquartile range; PCT, procalcitonin; WAZ, weight-for-age *Z* score.

of disease burden can also be translated into economic costs, which affect healthcare systems and also represent a relevant household economic burden, which is of special relevance in developing areas in which access to inexpensive treatments is difficult (Patil *et al.*, 2002; Rheingans *et al.*, 2012). These costs are also reflected in social inequities, with a trend towards lower expenditure related to diarrhoea in poorer households, which in some countries may often more frequently affect girls, and subsequently result in an increased risk of death (Rheingans *et al.*, 2012).

Regarding Morocco, in 2011, 132 000 children less than 5 years of age were reported to have different degrees of dehydration associated with diarrhoea. Of these, approximately 23 000 (17.4 %) were from the Rabat-Salé-Zemmour-Zair region, especially from urban areas (75.5 % of the cases). Additionally, at a national level, 7247 dysentery cases were reported in 2011, of which 320 were from the Rabat-Salé-Zemmour-Zair region, mostly (315 cases, 98.4%) from urban areas (Ministère de la Santé, 2012).

A series of pathogens, including bacteria, parasites and viruses, may act as the aetiological cause of this illness (Mandomando *et al.*, 2007; Vargas *et al.*, 2004). Nonetheless, the aetiological agents of diarrhoea vary greatly depending on the geographical origin. In addition, the clinical relevance of each specific pathogen also differs (Kotloff *et al.*, 2013; Lanata *et al.*, 2013; Pons *et al.*, 2013; Prère *et al.*, 2006) and, thus, a clear understanding of the prevalent locally specific aetiologies is essential for the design of specific prevention and control measures targeting the main causes.

Although some data on the aetiological causes of diarrhoea in some North African countries are available (Al-Gallas *et al.*, 2007; Hassine-Zaafrane *et al.*, 2011, 2013; Sdiri-Loulizi *et al.*, 2009, 2011), little is known about the aetiology and epidemiology of diarrhoea in Morocco. The latest estimates suggest that diarrhoea may be responsible for the death of 36 per 1000 live births annually in Morocco (UNICEF/WHO, 2009). However, the small amount of data available regarding the main aetiological causes of diarrhoea in Morocco are fragmented, and mainly focused on rotavirus, in relation to the introduction of the rotavirus vaccine (Rotarix) in the year 2010 (Benhafid *et al.*, 2012). Data regarding other pathogens are scarce and mostly outdated. The relevance of *Giardia intestinalis* and *Entamoeba histolytica* infections as a cause of diarrhoea in this country has also been shown. Thus, a report analysing 4285 cases of diarrhoea showed that these two parasites might altogether account for more than 50 % of positive parasite-associated cases (El Guamri *et al.*, 2009). Local data about the presence of diarrhoeagenic bacteria in different food products can also be found (Bennani *et al.*, 2011), and specific data regarding infections by *Salmonella* spp. have been published (Ammari *et al.*, 2009). However, a comprehensive description of the epidemiology and aetiology of diarrhoea in Morocco remains to be performed.

Thus, the main aim of this study was to describe the aetiology, epidemiology and clinical characteristics of the principal causes of acute infectious diarrhoea requiring hospitalization among children less than 5 years of age in a referral paediatric hospital in Rabat, Morocco.

METHODS

Site description. This prospective study was conducted in the Gastrointestinal Diseases and Emergency Departments at the Hôpital d'Enfants de Rabat (HER) in Rabat (Rabat-Salé-Zemmour-Zair region, Morocco). The HER is the only tertiary paediatric hospital in the Rabat-Salé-Zemmour-Zair region, but also attends infants from other Moroccan regions (especially from the north of the country). Thus, in 2011 the HER received 120 771 outpatient visits, with 18 471 hospital admissions (Ministère de la Santé, 2012).

In 2011, the population of the country was reportedly 32 187 000 inhabitants, 2 872 000 of whom were children (8.9 %) under the age of 5 years, and 506 000 children (1.6 %) were less than 1 year of age (Ministère de la Santé, 2012). The population of the Rabat-Salé-Zemmour-Zair region was 2 695 000 inhabitants, mostly in the urban area of Rabat (2 270 000 persons, 84.2 %), which included 225 000 children under 5 years of age (Ministère de la Santé, 2012).

Study population. The study included children >2 months and less than 5 years of age attending the HER from March 2011 to March 2012, with a primary diagnosis of acute diarrhoea, defined as three or more abnormally loose or liquid stools in the previous 24 h, having begun during the 7 days prior to admission to the hospital, with no other known cause of illness, and for whom diarrhoea was the principal cause of hospital admission. Diarrhoea cases due to chronic, ongoing, previously diagnosed gastrointestinal diseases were excluded. Likewise, outpatients were not included for not fulfilling the severity inclusion criteria.

Children fulfilling the inclusion criteria and whose parents had signed an informed consent underwent standardized procedures. Demographic, socio-economic and clinical data (including evolution during admission and outcome) were routinely collected following a standardized questionnaire and subsequently double entered using a program written in Filemaker Pro 12 (Filemaker). Treatment of the diarrhoea episodes and other related diagnoses was carried out according to national guidelines and decided by hospital clinicians. Antibiotic therapy was reassessed according to culture results and susceptibility patterns. The rotavirus vaccination status was established either by direct revision of vaccination documents or, in the absence of these documents, by asking the parents/guardians. The study protocol was approved by the Ethics Committees of the Hospital Clinic, University of Barcelona (Barcelona, Spain), and by the Institutional Review Board (Comité d'Éthique de la Recherché Biomédicale) of the Faculty of Medicine, University Mohamed V (Rabat, Morocco).

Case definitions. All case definitions were based on data obtained at admission from standardized study questionnaires. Fever was defined as an axillary temperature of $\geqslant$37.5 °C, and hyperpyrexia implied a temperature $\geqslant$39 °C (Guinovart *et al.*, 2008). Nutritional status was based on weight-for-age *Z* score (WAZ), calculated using the least mean square method and the 2000 Centers for Disease Control and Prevention Growth Reference (Kuczmarski *et al.*, 2002). Dehydration status was established according to standard World Health Organization criteria (WHO, 2005). Dehydration was considered moderate when estimated between 5–10 % and severe if >10 % (Stoll *et al.*, 1982). The minimum community-based incidence rates of diarrhoea were estimated using the Rabat-Salé-Zemmour-Zair region population as described elsewhere (Kotloff *et al.*, 2013).

Sample collection. At enrolment at least 5 ml or 5 g stool was collected from each patient by either collection in a waxed-cardboard

container at the time of defecation or from the diaper if applicable. All samples were processed within a maximum of 12 h after collection. Additionally, 1 to 2 ml of venous whole blood was collected on admission for biomarker evaluations.

Biomarker determinations. Procalcitonin (PCT) and C-reactive protein (CRP) levels were determined using miniVIDAS (bioMérieux) and Microlab 300, respectively.

Bacterial culture. In order to search for the presence of *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Vibrio cholerae*, *Yersinia* spp., *Aeromonas* spp. and diarrhoeagenic *Escherichia coli,* faeces were cultured in different media [MacConkey, Campylobacter agar, blood agar, Salmonella Shigella (SS) agar, xylose lysine deoxycholate (XLD) agar, cefsulodin–irgasan–novobiocin (CIN) agar and thiosulfate–citrate–bile salts–sucrose (TCBS) agar]. Bacterial isolates were identified based on growth in the aforementioned media (e.g. *Salmonella* spp. and *Shigella* spp. were recovered from MacConkey, XLD and SS agar; *E. coli* from MacConkey agar; *Campylobacter* spp. from Campylobacter agar; while TCBS was used to detect the presence of *Vibrio* spp., CIN to isolate *Yersinia* spp., and blood agar to isolate *Aeromonas* spp.), and by colony morphology, conventional biochemical techniques (Murray *et al*., 2007) or an automated system (Phoenix 100; Becton Dickinson).

Detection of diarrhoeagenic *E. coli* strains. Diarrhoeagenic strains of *E. coli* [enteroaggregative *E. coli* (EAEC); enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); diffusely adherent *E. coli* (DAEC); enteroinvasive *E. coli* (EIEC); enterohaemorrhagic *E. coli* (EHEC)] were detected by Real time multiplex PCR using the primers and methodology described by Guion *et al.* (2008).

Parasite identification. The faecal material obtained from the patients was concentrated using the Ritchie technique, and then stained following the modified Ziehl–Neelsen staining procedure in order to detect *Cryptosporidium* spp. (Bailenger, 1973; Tligui & Agoumi, 2006). The presence of *Giardia* spp. and *Entamoeba histolytica* was determined by microscopy using the Bailenger technique (Bailenger, 1973; Bourée, 1994).

Virus detection. Nucleic acid for viral studies was extracted using a commercial kit (MagMax total nucleic acid isolation kit; Applied Biosystems). Detection and genotyping of rotavirus was performed following the procedures of Rodríguez-Díaz *et al.* (2009). Detection of sapovirus, norovirus and astrovirus was carried out using the primers described by Yan *et al.* (2003) with a multiplex Reverse transcriptase (RT)-PCR using the standard conditions described in a commercial kit (Superscript III one step RT-PCR; Invitrogen, Genome Biotechnologies). The presence of hepatitis A was established in a monoplex RT-PCR as described elsewhere (Sánchez *et al.* 2002).

RESULTS

During the 13-month long study period, 852 out of the 11 799 children (7.2 %) attending the Paediatric Emergency Department of the HER in Rabat presented with acute gastro-intestinal symptoms, resulting in a minimum community-based incidence rate of diarrhoea in the region of Rabat-Salé-Zemmour-Zair of 0.35 episodes per 100 children per year. Of these, 720 (84.5 %) were seen as outpatients and did not require admission, while 132 children fulfilled the enrolment criteria and were recruited for the study showing a minimum community-based incidence rate of moderate-to-severe diarrhoea in the Rabat-Salé-Zemmour-Zair region of 0.06 episodes per 100 children per year. A total of 10 patients were discharged prior to obtaining all the necessary samples and, thus, 122 children were finally included in the analysis (Fig. 1).

Diarrhoea cases were predominantly seen (73/122; 59.8 %) during the cold season (January–March). The mean age of the children recruited was 16.5 months (range 2.4 to 54.2 months), with a predominance of males (53.3 %). Diarrhoea episodes had a median duration of 4 days [interquartile

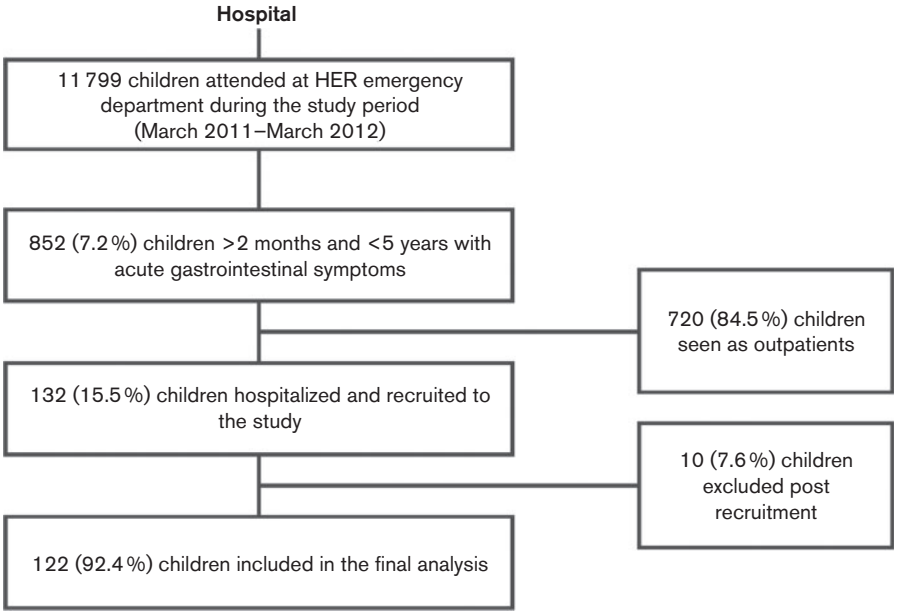

Fig. 1. Study profile.

range (IQR) 1–5], and 103 (84.4 %) children presented fever and 108 (88.5 %) vomiting. Parents of 29 out of the 122 (23.8 %) patients referred pre-admission usage of antibiotics, mainly β-lactam antibiotics (12 cases) and co-trimoxazole (12 cases). Malnutrition was common among the study population, with over half of the patients recruited (52.4 %) showing some degree of malnutrition (WAZ <-1) and almost 15 % of the patients being severely malnourished (WAZ <-3). Other relevant clinical and demographic data are presented in Table 1.

A total of 12 (9.8 %) children were referred to the intensive care unit (ICU) at the HER, while two, presenting EAEC, and EAEC plus a *Shigella sonnei* infection, developed a HUS. Six out of these twelve children (50 %) died, representing 4.9 % of the total number of children recruited. In four out of these six cases, EAEC infection (one coexisting with an astrovirus) was identified. The final

Table 1. Clinical and demographic data of patients included in the study

Relevant parameter	Value
Age (months)	
Mean (SD)	16.5 (11.5)
Range	2.4–54.2
Sex	
Male	65/122 (53.3 %)
Female	57/122 (46.7 %)
No. of referred stools day^{-1}	
Mean	6.1
Range	3–15
Duration of diarrhoea episode: median (IQR)	4 (2–5)
Blood in faeces	3/122 (2.5 %)
Mucus in faeces	79/102 (77.5 %)
Fever	103/122 (84.4 %)
Complications	
Transfer to ICU	12/122 (9.8 %)
HUS	2/122 (1.6 %)
Death	6/122 (4.9 %)
Vomiting	
Total no.	108/122 (88.5 %)
Mean no. of episodes	5.1
Duration (days): median (IQR)	3 (2–5)
Breastfeeding/feeding difficulty	95/122 (77.9 %)
Paleness	62/103 (60.2 %)
Dehydration	
Total no.	121/122 (99.2 %)
Mild	51/121 (42.1 %)
Moderate	64/121 (52.9 %)
Severe	6/121 (5.0)
Nutritional status	
WAZ score [mean (SD)]	−1.1 (1.8)
Malnutrition (WAZ <-1)	54/103 (52.4 %)
Haemoglobin (g dl^{-1})	
Mean	11.4
Range	6.4–14.8

Table 2. Clinical descriptions of severe cases of diarrhoea resulting in death

Patient	Age (months)	Medical insurance	No. of days with diarrhoea	No. of stools in 24 h	Convulsions	Dehydration	ATB	Creatinine	ICU	No. of days of hospitalization	HUS	Micro-organism	Final diagnosis
DR0007	23.4	No	3	10	Yes	Severe	No	NA	Yes	2	No	EAEC/DAEC	Acute gastroenteritis
DR0009	21. 7	No	3	4	Yes	Moderate	No	0.37	Yes	2	No	None	Acute gastroenteritis
DR0013	4.4	No	4	NA	Yes	Moderate	No	0.7	Yes	0	No	Astrovirus /EAEC	Acute gastroenteritis
DR0016	3.0	No	3	5	No	Moderate	No	2.94	No	14	No	EAEC	Renal failure
DR0020	24.9	No	1	6	No	NA	Yes	1.21	Yes	0	No	EAEC	DIC
DR0054	8	No	5	4	Yes	Severe	No	NA	No	1	No	NA	Acute gastroenteritis

ATB, Pre-admission antibiotic intake; DIC, disseminated intravascular coagulation; NA, not available.

diagnosis in patients who died, obtained by the study clinicians after review of the whole hospitalization file, corresponded to acute gastroenteritis/diarrhoea (four cases), acute renal failure (one patient with a prolonged hospitalization of 14 days) and disseminated intravascular coagulation (one case). Importantly, neurological abnormalities (convulsions and/or impaired consciousness) were of note during these diarrhoea episodes ending in death (Table 2).

Regarding specific infection biomarkers, 57.0 and 29.0 % of the patients with available results (n=100) presented increased levels of PCT or CRP, respectively. The mean PCT value was significantly higher in patients with bacterial infection (18.0) compared to the mean value in patients with viral infection (2.0; $P<0.001$). However, the mean CRP value was comparable in both bacterial and viral infections, being below the threshold defined as elevated in both cases (0.05 g l^{-1}). Interestingly, patients in whom neither viruses nor bacteria were isolated from stools showed the highest CRP and PCT levels (Table 3).

At least one pathogen was isolated in 89 out of the 122 faecal samples (73.0 %). The most frequent aetiological agents were: diarrhoeagenic *E. coli* (71 isolates, 58.2 %); rotavirus (21, 17.2 %) belonging to genotypes G1P8 (16, 76.2 %), G3P9 (4, 19.0 %) and G8P9 (1, 4.8 %); and *Shigella* spp. (8, 6.5 %) (Table 4). Co-infections were frequent and present in 25 (20.5 %) of the patients, including rotavirus and *E. coli* (EAEC) (7 cases, 28 %), and rotavirus and *E. coli* (DAEC) (3 cases, 12 %), as the most frequent combinations, while three or more pathogens were recovered in another two patients (Table 5).

The most frequent diarrhoeagenic *E. coli* was EAEC (47 cases, 38.5 %), followed by DAEC (15, 12.3 %), EPEC (7, 5.7 %) and ETEC (2, 1.6 %), and two (1.6 %) isolates presented both the EAEC and DAEC characteristics. Neither EHEC nor EIEC were isolated.

A total of 38 out of the 122 children (31.1 %) had received at least one dose of the currently implemented rotavirus vaccine (Rotarix). Six had received three doses, while seventeen had received two doses and fifteen reported to have received only one dose of the vaccine. The remaining 84 children recruited were not vaccinated or vaccination data were not documented. Rotaviruses in faeces were mainly recovered (14 cases, 66.7 %) from children apparently not vaccinated or for whom data were unavailable. However, rotavirus infections were also detected in children with partial or complete rotavirus vaccination: two cases in patients having received one dose; five further cases in children having received two doses; and one case in a child reporting three doses (Table 6). Finally, rotavirus infections seemed to show a clear seasonality, being mostly detected during the cold season (Fig. 2).

DISCUSSION

Diarrhoea remains a relevant cause of childhood morbidity and mortality in Morocco, as previously suggested by the scarce reports available from this country or from the Maghreb area (Bourrous *et al.*, 2010; INSPA, 2005). Indeed, while diarrhoea-related admissions were relatively uncommon in the hospital (HER) (only 122 cases during a 13 month period), mortality associated with this syndrome in Rabat was high (6 deaths, 4.9 %), especially when compared to the recent results of a large multicentre study on the global aetiology of diarrhoea showing a varying range of diarrhoea-attributable case fatality rates (from 0.13 % in India to 7.5 % in Mozambique, with a mean of 2 %) (Kotloff *et al.*, 2013). In four out of these six deaths, diarrhoeagenic *E. coli* (three EAEC, one EAEC/DAEC) was detected in faeces. Despite attributing causality to these microbiological findings, the determination of the precise cause of death in these patients is challenging and may be inappropriate without adequate post-mortem confirmation, and without thorough exclusion of other potential co-morbidities. However, the role of diarrhoeagenic *E. coli* as a cause of child mortality has been robustly documented and reported elsewhere (Kotloff *et al.*, 2013; Lanata *et al.*, 2013; Nataro *et al.* 1998).

Two cases of HUS were detected as severe complications among children admitted with acute diarrhoea. HUS is a serious complication that is often associated with the presence of specific pathogens such as EHEC or *Shigella* spp. (Khan *et al.*, 2013; Fischer Walker *et al.*, 2012). In our series, no EHEC isolate was found, and in both HUS cases an EAEC isolate was detected, one being associated with *S. sonnei* co-infection. Although *Shigella dysenteriae* type 1 is, by far, the member of the *Shigella* genus most often implicated in the development of HUS (Fischer Walker *et al.*, 2012), a recent report from Bangladesh confirmed the potential of *S. sonnei* as an aetiological trigger for HUS (Khan *et al.*, 2013). Despite a recent outbreak in Germany

Table 3. Laboratory findings according to micro-organisms detected in stools

Biomarker	Bacteria	Virus	Mixed bacteria/virus	No pathogen detected
PCT (ng ml^{-1})				
Mean	18	1.98	18.7	42.49
Range	0.05–243.7	0.06–16.18	0–370	0–300
C-reactive protein (mg dl^{-1})				
Mean	2.5	2.14	2.3	5.0
Range	0.1–19.2	0.1–7.7	0–19.2	0–35.9

Table 4. Micro-organisms detected

Micro-organism	No. of cases (%)
Bacteria	
EAEC	47 (38.5)
DAEC	15 (12.3)
EPEC	7 (5.7)
ETEC	2 (1.6)
EAEC/DAEC	2 (1.6)
Shigella spp.	9 (7.4)
Salmonella spp.	5 (4.1)
Campylobacter spp.	5 (4.1)
Protozoa	
Giardia intestinalis	1 (0.8)
Entamoeba histolytica	1 (0.8)
Virus	
Rotavirus	21 (17.2)
Astrovirus	6 (4.9)
Hepatitis A	1 (0.8)
Norovirus	1 (0.8)

EAEC/DAEC, Micro-organisms presenting EAEC and DAEC mixed characteristics.

involving EHEC/EAEC isolates (Aurass *et al.*, 2011), to our knowledge, the role of EAEC as a cause of HUS remains undescribed and, thus, no direct association may be extrapolated from the current data. Despite data regarding HUS in middle- and low-income countries being scarce, this syndrome is the most relevant cause of acute kidney injury among paediatric populations, especially affecting young children (Hofer *et al.*, 2014). Regarding Morocco, HUS has been described as the second most frequent cause of acute infantile renal failure (Bourquia *et al.*, 2002) and, similar to other diarrhoea complications, this syndrome contributes to long-term diarrhoea-related morbidity.

Table 5. Common co-infections in patients with acute diarrhoea

Coinfection	No. of cases
Rotavirus–*E. coli* (EAEC)	7
Rotavirus–*E. coli* (DAEC)	3
E. coli (EAEC)–*Shigella*	3
E. coli (EAEC)–*Campylobacter*	3
E. coli (EAEC)–*Salmonella*	2
Astrovirus–*E .coli* (EAEC)	2
Rotavirus–*E. coli* (EPEC)	2
Astrovirus–*E. coli* (DAEC)	1
Norovirus–*E. coli* (EAEC)	1
E. coli (DAEC)–*Campylobacter*	1
Hepatitis A–*E. coli* (EPEC)	1
Rotavirus–*E. coli* (EAEC)–*Campylobacter*	1
Rotavirus–*E. coli* (DAEC)–*Salmonella*	1

Table 6. Rotavirus genotypes and vaccination status of rotavirus-associated diarrhoeal episodes

Genotype	Vaccination status			Rotarix
	0 doses	1 dose	2 or 3 doses	
G1P8	12	1	3	+
G3P9	1	0	3	−
G8P9	1	0	0	−

+, Serotype covered by Rotarix; −, serotype not covered by Rotarix.

Thus, in some cases HUS may lead to the development of different chronic problems, such as long-term hypertension, diabetes mellitus or neurological sequelae among other adverse extrarenal outcomes, as well as different degrees of long-term impairment in renal function. Indeed, around 5 % of cases develop chronic renal failure requiring dialysis or kidney transplantation (Spinale *et al.* 2013), which in low-income countries may lead to death due to the lack of adequate treatment.

As anticipated, and in accordance with previous studies having shown the potential of PCT as a predictor of bacterial blood infections (Díez-Padrisa *et al.*, 2012), PCT levels were significantly higher amongst bacteria-related diarrhoea cases compared to virus-related diarrhoeal episodes. The higher levels of PCT observed in diarrhoea cases in which no specific pathogen was isolated suggest the presence of unidentified bacteria/parasites more than the presence of viruses.

A low number of parasitic infections have been described in studies of the aetiology of diarrhoea in the Maghreb area (Al-Gallas *et al.*, 2007). This low prevalence was confirmed in our series, in which only two parasitic infections (*G. intestinalis*, *Entamoeba histolytica*) were detected, being lower than that observed in a previous study performed in the same hospital with identical methodologies, in which a total of 10 *G. intestinalis* isolates were detected in a series of 63 children (15.9 %) with stature–ponderal delay (Oudaïna *et al.* 2009). A possible explanation for the difference in positivity between the two studies may be that diarrhoea associated with parasites does not require hospitalization, and no parasites were detected in the present series. However, it is likely that the use of molecular techniques for parasite detection would result in a higher detection capacity.

The vast majority of the cases of diarrhoea described in this report were related to bacterial infections, predominantly caused by diarrhoeagenic *E. coli*, particularly due to the EAEC and DAEC pathotypes, but also by *Shigella* spp., *Salmonella* spp. and *Campylobacter* spp. The role of EAEC as a relevant cause of paediatric diarrhoea has been described worldwide (Kotloff *et al.*, 2013; Mandomando *et al.*, 2007; Ochoa *et al.* 2009; Vargas *et al.*, 2004). Interestingly, the second most frequent diarrhoeagenic

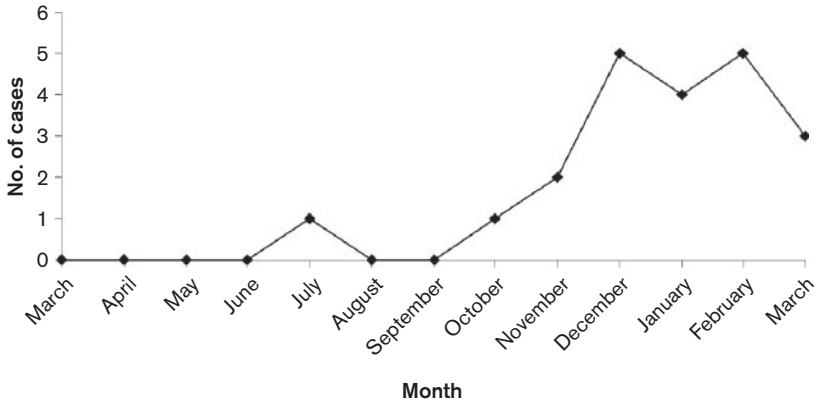

Fig. 2. Rotavirus detection in patients with acute diarrhoea according to seasonality (period March 2011–March 2012).

pathotype of *E. coli* isolated was neither EPEC nor ETEC, similar to previous studies (Mandomando *et al.*, 2007; Vargas *et al.*, 2004), but rather DAEC. Two *E. coli* isolates presenting mixed characteristics (mixed EAEC/DAEC pathotypes) were detected. The presence of diarrhoeagenic isolates presenting mixed characteristics of two different pathotypes is not a new finding (Aurass *et al.*, 2011; Ruiz *et al.*, 2008). Furthermore, the presence of EAEC/DAEC has recently been described in South America (García *et al.*, 2011). This fact is of special concern because it might reflect either the intercontinental spread of new mixed pathotypes, or their parallel evolution in geographically distant areas. The public-health risk of such mixed pathotypes was clearly established in the recent Germany EHEC/EAEC outbreak, which resulted in approximately 4000 infected persons including more than 900 cases of HUS and 59 deaths (Karch *et al.*, 2012).

Rotavirus, followed by astrovirus, accounted for the majority of viral-related diarrhoeal episodes. Rotavirus infections were essentially recovered during the coldest months, as described elsewhere (Benhafid *et al.* 2013), even in the same geographical area (Hassine-Zaafrane *et al.*, 2011). Rotavirus was the most frequent virus involved in the development of cases of diarrhoea, and ranking as the specific second cause of diarrhoea after EAEC isolates. Three different genotypes were detected: G1P8, G3P9 and G8P8. While G3P9 and G8P8 are not included in the recently introduced rotavirus vaccine in Morocco, G1P8, the most prevalent genotype detected, is adequately covered by this vaccine (Benhafid *et al.* 2013). The G1P8 genotype was detected in four children partially or fully vaccinated. This might be explained by the fact that a low incidence of new cases would be expected in children adequately vaccinated.

Although the relevant role of rotavirus and the proportion of cases attributable to astrovirus are in agreement with what has been previously described in the north of Africa (Sdiri-Loulizi *et al.*, 2009), the low incidence of norovirus is in clear disagreement with previous data in the Maghreb area (Hassine-Zaafrane *et al.*, 2013). Thus, no clear reason is available to explain the lack of norovirus as a cause of paediatric diarrhoea in our series. However, sapovirus has been described as a common cause of mild-to-moderate diarrhoea, usually not requiring hospitalization, in Tunisian children (Sdiri-Loulizi *et al.*, 2011), which may explain why so few cases of sapovirus were detected among our series of moderate-to-severe patients with diarrhoea requiring admission.

To the best of our knowledge, this is the first report providing a comprehensive assessment of the aetiological causes of severe paediatric diarrhoea in Morocco. Despite some limitations, such as the inability to detect some recently described emerging pathogens, e.g. *Campylobacter concisus* (Nielsen *et al.*, 2013), this study sets the basis for further research regarding paediatric diarrhoea in the area, and advocates for the establishment of adequate hospital-based microbiological surveillance systems. The low-to-moderate burden of diarrhoea-related admissions among Moroccan children, as detected in the HER of Rabat poses, however, a major public-health problem, particularly due to the unexpectedly high associated case fatality rates. We have demonstrated the relevant role of diarrhoeagenic *E. coli* and rotavirus as the two main causes of severe diarrhoea in this area, with the lower contribution of other pathogens such as norovirus or parasites being of note. These data call for the implementation of better surveillance and prevention programmes, as well as improvement in the early recognition and management of potentially life-threatening episodes of diarrhoea.

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