VICO: A public health surveillance system for bacterial, parasitic, and viral causes of diarrhea, respiratory disease and acute febrile illness in Guatemala

(Thirteenth Amendment, November 2013)

Protocol summary

An integrated surveillance system for diarrheal and respiratory disease (influenza-like illnesses [ILI], pneumonia and other severe lower respiratory diseases), and acute febrile illness (AFI) at multiple levels of the government health services, , will be established in the Departments of Santa Rosa and Quetzaltenango, in Guatemala to provide information on burden and cause of disease and lay the foundations for early warning for outbreaks, epidemics and emerging infections. Patients admitted to a participating hospital or examined at any of the participating Health Centers or Health Posts will be screened to determine whether they meet the case definitions.. Patients who meet case definitions and agree to participate in the project will be asked for illness, demographic and risk factor information and then have appropriate samples taken to determine the etiology of their infections. Samples will be processed in the laboratories of the participating hospitals, the Guatemalan National Health Laboratory, the Universidad del Valle de Guatemala (UVG) and at the laboratories of the US Centers for Disease Control and Prevention in Atlanta, GA, and Fort Collins, CO. Annual age-specific incidence rates for these syndromes will be calculated and etiologies will be identified by an array of diagnostic tests. Risk factors will be identified by comparison with population-level information collected as part of a household survey and with data collected from patients with other syndromes. This project will be conducted in conjunction with the Guatemalan Ministry of Public Health and Social Assistance (MSPAS) who will use these data for the purposes of planning and assessing interventions. Results will also be published in peer-reviewed literature.

Roles of investigators

John McCracken, ScD Epidemiologist of the Emerging Infecious Diseases Unit, Center for Health Studies, UVG, is the Principal Investigator for this project.

All co-investigators in this project will be involved in analysis and interpretation of data and the preparation of reports and manuscripts. Specific duties follow.

<u>Diarrheal diseases</u>:

The following individuals will be responsible for developing protocols related to the surveillance and diagnosis of mild and severe cases of diarrheal disease.

Enteric Diseases Epidemiology Branch and the Enteric Diseases Laboratory Response Branch Division of Bacterial and Mycotic Diseases, National Center for Zoonotic, Vector-borne and Enteric Diseases, CDC

Eric Mintz MD
Cheryl Bopp MS
Michele ParsonsMS
Debora Talkinton MS
Olga Henao MPH PhD

Epidemiology Branch, Division of Viral Diseases, National Center for Immunization and Respiratory Diseases (Proposed), CDC

Umesh Parashar MD
Jon Gentsch PhD
Parasitic Branch, Division of Parasitic Diseases, National Center for Zoonotic, Vector-borne and Enteric Diseases; CDC
Vitalino Cama PhD

Additionally, the following individuals from the Division of Epidemiology, University of California, Berkeley, will be involved in the analysis of diarrheal surveillance data:
John M. Colford, Jr. MD PhD
Ben Arnold, PhD

Respiratory diseases

The following individuals will be responsible for developing protocols related to the surveillance and diagnosis of mild and severe cases of influenza-like illnesses, pneumonia and other lower respiratory tract infections:

Respiratory Diseases Branch, Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases
Cynthia Whitney MD
Stephanie Schrag PhD
Stephanie Schwartz, MS
Jennifer Rabke Verani, MD MPH
Streptococcus Laboratory, CDC Division of Bacterial Diseases
Maria da Gloria Carvalho, Ph.D.

Epidemiology Branch, Division of Viral Diseases, National Center for Immunization and Respiratory Diseases
Dean Erdman, DPH
Eileen Schneider, MD

Epidemiology Branch, Influenza Diseases, National Center for Immunization and Respiratory Diseases
Marc-Alain Widdowson, MD

Febrile illness

The following individuals will be responsible for developing protocols related to the surveillance and diagnosis of febrile illnesses of unknown origin:

Arbovirus Disease Branch, Division of Vector-Borne Infectious Diseases, National Center for Zoonotic, Vector-borne and Enteric Diseases (Proposed)
Ann Powers PhD

Emily Zielinski-Gutierrez MPH DrPH
Jim Sejvar MD

Division of Viral and Rickettsial Diseases, National Center for Zoonotic, Vector-Borne and Enteric Diseases, CCID, CDC
Gregory Dasch, PhD
Marta Guerra, DVM
Amanda Loftis, DVM

Center for Health Studies, Universidad del Valle de Guatemala FWA00001902
Celia Cordon-Rosales Lic.
Pamela Pennington PhD

Design and implementation of the surveillance system

The following individuals will be responsible for contributing to the design of the integrated surveillance system, supervising field activities, including data collection, and supervising diagnostics and quality control.

International Emerging Infections Program, Global Disease Detection, Center for Global Health
Joe Bryan, MD

Center for Health Studies, Universidad del Valle de Guatemala, FWA 00001902
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Sergio Vinicio MD

Manuel Sagastume, MD
Francisco Ardon, MD
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Funding mechanism

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Introduction

Background

Guatemala is a developing country with both high under-5 mortality (30 per 1000 live births) [1]and high adult mortality (282 and 155 per 1000 people aged 15 to 60 for males and females, respectively) [2]; life expectancy at birth for both sexes combined is 71 years [1]. Infectious diseases constitute a major cause of death and disability in Guatemala, accounting for 32% of all disability-adjusted life-years (DALYs) lost and 41% of all deaths in 2002 (WHO,http:// www.who.int/healthinfo/global_burden_disease/en/index.html).

Diarrhea and respiratory illnesses are the major infectious causes of death in Guatemala. Diarrhea was responsible for 3,400 deaths (4% of total) and respiratory diseases for 5,900 deaths (7% of total) in 2002 (http://www.who.int/healthinfo/global_burden_disease/en/index.html). The proportions of DALYs due to diarrhea and respiratory diseases were similar to their proportions of deaths. Diarrhea and respiratory diseases are also the top 2 reasons for seeking care at a government health facility in Guatemala.

Despite the apparent high mortality and disability due to these syndromes, these estimates of mortality and disability from government statistics are likely to under-report the burden of disease as population-based data are lacking. Additionally, the etiologies of diarrhea and respiratory disease in Guatemala, as in most developing countries, are poorly understood. Inconsistent case definitions,[3, 4] limited availability of diagnostic testing in the country and limited surveillance data contribute to the poor state of knowledge regarding two of the most important disease syndromes in Guatemala. Without a better measurement of the burden of disease caused by these syndromes and knowledge of the causal organisms, it will not be possible to reduce the incidence or prevalence of diarrhea and respiratory disease and their associated mortality rates. In addition, new intervention strategies, such as vaccines or clean water systems, cannot be evaluated without better data.

Detection and assessment of the burden of enteric infections through an in-depth surveillance system is necessary to increase sensitivity and efficiency of case ascertainment, while also maintaining the comparability over time of data on disease occurrence for evaluation of burden, long-term trends, unexpected increases, and impact evaluation of public health programs.

As the global community prepares for a potential influenza pandemic, it is clear that there is little known about either the incidence of seasonal influenza in Guatemala and the Central American region or the circulating strains. This information is critical to preparing surveillance systems for pandemic preparedness.

While some of the potential causes of acute febrile illness (AFI), such as dengue and leptospirosis, are included in the list of notifiable diseases in Guatemala, case identification is limited by the lack of diagnostic facilities and hence lack of recognition. The presence of competent vectors and the broad ecological similarities between Central and North America suggest that the widespread infections seen in North America could present a similar threat to Guatemala. The introduction of WNV to North America and its rapid spread throughout the western hemisphere provide a vivid example of the ability of an emerging virus to cause a significant public health impact in a new environment. An effective febrile illness surveillance system would be a sensitive method for early detection of additional emerging infections.

Leptospirosis, caused by the spirochete bacterium *Leptospira interrogans,* causes a febrile illness accompanied by headache, chills, vomiting, eye inflammation, and muscle aches. In more severe cases, the illness can result in liver damage and jaundice, kidney failure, and internal bleeding. Large outbreaks have occurred in multiple countries following hurricanes, as was experienced in 1998 in the aftermath of Hurricane Mitch. Previous experience after flooding has demonstrated various risk factors and sources of infection, and epidemic leptospirosis accompanied by pulmonary hemorrhage has been reported in Nicaragua. The first successful studies of chemoprophylaxis against leptospirosis were conducted in Central America, in Panama during the early 1980s. Although leptospirosis is endemic in much of the world, including Central America, the burden of this disease in Guatemala has not been defined.

In addition to the burden of disease due to diarrhea, respiratory and acute febrile diseases, in the general population of Guatemala, the pathogens responsible may also be causes of outbreaks and pandemics. Outbreaks of diarrheal disease traced to Guatemalan food products have occurred in the United States. While there have yet been no outbreaks of respiratory or febrile illnesses in the US attributed to sources in Guatemala, it cannot be predicted where new infections will emerge. The 2003 SARS-coronavirus global outbreak and 2009 pandemic influenza H1N1 highlight the need for rapid detection and response to outbreaks of severe respiratory illness caused by unknown etiologies in multiple regions of the world. The proximity of Guatemala to the US, in addition to the number of Guatemalan migrants who cross into the US each year, suggest that it is an appropriate location from which to conduct population-based surveillance to provide the basis for establishment of an early warning system for infections with the potential for causing outbreaks or trans-national epidemics.

Project justification

The establishment of laboratory-enhanced, population-based surveillance for diarrhea, neurologic disease, respiratory disease and febrile illnesses of unknown origin based in the Guatemalan MSPAS health services will provide accurate incidence rates of these syndromes and important information on the etiologies of disease. The ongoing collection of this information will assist the MSPAS in designing interventions, allow monitoring of drug resistance, permit evaluation of new interventions and provide the basis for development of an

early warning system. Calculating accurate incidence rates will also enable comparison between surveillance results from Guatemala and data from other CDC Global Disease Detection (GDD) sites. Finally, an ongoing surveillance system coupled with good laboratory diagnostics will permit the early identification and rapid response to outbreaks, epidemics and emerging infections.

Potential use of project findings

Project findings will be communicated regularly to the directors of the participating Health Areas, Health Districts, and National Hospitals, the MSPAS's health services directorate, and the Guatemalan National Center for Epidemiology. These reports will help the MSPAS to set priorities for allocation of funds, design and evaluate disease-specific interventions (new vaccines, public health messages, etc.) and inform clinical guidelines (*i.e.* first and second-line antibiotics). These data may lead to the identification of new infectious agents and enable early detection of outbreaks and epidemics. This long-term, systematic surveillance of infectious diseases will provide data for representation of Guatemala and Central America in projects estimating the regional and global trends and burdens of disease due to key syndromes and pathogens and lead to valuable scientific publications.

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Project design and location

The project will collect information on incident cases of hospitalized diarrhea, se respiratory disease and acute febrile illness of unknown origin in 2 departments in Guatemala (Santa Rosa, and Quetzaltenango), as well as incident cases of ambulatory diarrhea, influenza-like illness, and acute febrile illness of unknown origin in one municipality (like counties) in each department. Laboratory testing of specimens will occur in the laboratories of the participating national hospitals, at the laboratories of the UVG in Guatemala City, and at CDC labs in Atlanta and Fort Collins, and NAMRU-6 in Perú (for further testing on certain enteric pathogens only). Data analysis will take place at Universidad del Valle de Guatemala, US Centers for Disease Control and Prevention in Atlanta, GA, and University of California, Berkeley.

Objectives

The overall objective of this project is to establish baseline levels of disease due to diarrhea, respiratory illness, and febrile illness and to identify the main causes of these disease syndromes. These data will be used to assist with outbreak investigations, implement and evaluate interventions, guide public health policy and develop an early warning system. The surveillance system will act as a platform for potential studies.

Specific sub-objectives relate to each syndrome:

Diarrhea

1. Determine the age-specific incidence of diarrhea in outpatients less than five years of age;
2. Determine the age-specific incidence of hospitalized diarrhea cases less than five years of age;
3. Determine the proportion of diarrhea cases due to viral, bacterial, and parasitic pathogens (see Appendix C);
4. Measure resistance of bacterial pathogens to available antibiotics;
5. Type rotavirus strains (CDC, Atlanta) to assess potential preventability via vaccination;
6. Document and identify potentially modifiable personal, household and community risk factors (such as sanitation coverage and population density) for diarrhea and enteric infections, and estimate risks of infection to compare overall incidence rates and enteric pathogen profiles based on risk factors;
7. Detect early increases in the incidence of diarrhea that may signal an outbreak or epidemic to initiate investigative activities and prevention measures.
8. Create a repository of specimens for future tests.

Pneumonia

1. Determine the age-specific incidence of hospitalized community-acquired pneumonia;
2. Determine the major etiologies of hospitalized, community-acquired pneumonia;
3. Determine the etiology of severe respiratory disease in hospitalized adult patients using the Taqman Array Card.
4. Document and identify potentially modifiable personal, household and community risk factors (such as vaccination status, household air pollution exposure, crowding, and nutritional status) for respiratory infections.
5. Detect early increases in the incidence of community-acquired pneumonia that may signal an outbreak or epidemic to initiate investigations and appropriate control measures;

Influenza-like illness (ILI)

1. Describe the seasonality of influenza viruses in Guatemala;
2. Estimate the burden of influenza like illnesses that leads to clinic visitation;
3. Characterize influenza viral strains circulating in Guatemala and determine susceptibility to antiviral agents

Acute febrile illness (AFI)

1. Determine the age-specific incidence of AFI in patients who are admitted to the national hospitals and ambulatory cases that present to the health centers;
2. Determine the etiology of AFI;
3. Determine the geographic distribution of cases.

Methods

General approach

A prospective surveillance system with associated laboratory diagnostics will be established in 2 departments (Santa Rosa, and Quetzaltenango) in National Hospitals and the Health Centers and Health Posts in selected municipios in Santa Rosa and Quetzaltenango.

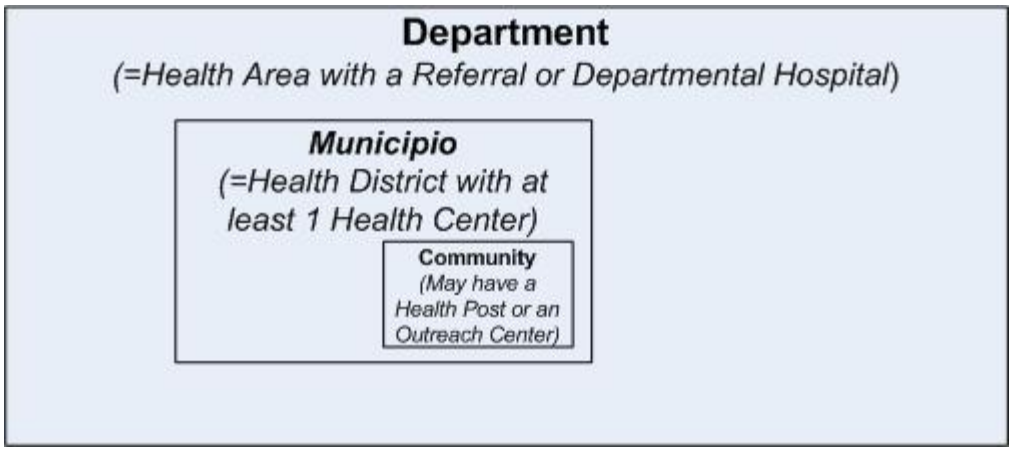
At the hospital, paid surveillance staff will actively search the emergency room, inpatient logs and laboratory and radiology registers to identify patients admitted with symptoms suggesting diarrhea, respiratory disease or acute febrile illness (AFI). After obtaining verbal, informed consent from patients or their caregivers, clinical information will be gathered to determine whether patients meet inclusion and exclusion criteria. Those who satisfy these criteria will be admitted into the project after giving written, informed consent, and appropriate biological samples, demographic, risk factor and health history information will be taken to assist in the diagnosis of the etiologic agent of their disease.

At the Health Center, patients will be directed to project staff if they present with symptoms of diarrhea, ILI or AFI. After receiving verbal, informed consent, paid surveillance staff will interview patients to determine if they meet inclusion and exclusion criteria. Patients who are admitted to the project will need to provide written consent before being interviewed for demographic, risk factor and health history information and appropriate biological samples will be taken. At the Health Post, patients with diarrhea and ILI will be asked for written, informed consent to participate in the project and will then be interviewed for demographic, risk factor and health history information and appropriate biological samples will be requested.

Laboratory diagnostics will be conducted at various laboratories depending upon the complexity and availability of the diagnostic methods (see laboratory methods section below). Results will be provided to the participating health facilities and the MSPAS on a quarterly basis.

Project setting

Guatemala is divided into 23 administrative departments, each of which is sub-divided into approximately 10-14 municipalities. Each department roughly corresponds to a “health area” that is responsible for all public health activities in its catchment area. Public health care in Guatemala is provided through a system of regional and departmental hospitals, health centers,



health posts, outreach centers and NGOs. There is generally one regional (referral) or departmental hospital per health area; each municipality corresponds to a "health district" and has at least 1 health center. Health centers are typically staffed by a physician and several nurses. Each municipality has several health posts, which are clinics located in outlying communities and staffed by nurses. Outreach centers are designed to provide care to the population that does not have access to health posts. Such centers may be visited by staff from the health centers and health posts, or may run by NGOs.

Project population

The Department of Santa Rosa (14.16°N, 90.48°W), located south of Guatemala City (Figure 1), has a population of 346,590 (2011) in an area of 3,164 km^2.[5] Elevation ranges from sea level on the Pacific Coast to 1,945 m at the top of the volcano Tecuamburro. The department is divided administratively into 14 municipalities (*municipios*)The department has one hospital located in Cuilapa,10 health centers, and 54 health posts.

The population of the Department of Santa Rosa is 65% rural and 35% urban and almost equally divided between men and women. Approximately 85% of the population is non-indigenous and 91% speak Spanish as their first language. More than a quarter of the population has had no schooling and 27% are unable to read or write (slightly more females, 28%, than males, 25%, are illiterate). The age distribution is as follows:

Age group	Percentage of total population
0-4 years	15%
5-9 years	14%
10-14 years	13%
15-19 years	11%

20-29 years	17%
30-39 years	10%
40-49 years	7%
50-59 years	5%
60 years +	8%

The incidence of diarrheal diseases in the department in 2012 was 4,681 per 100,000 residents [6]; the incidence of pneumonia and bronchopneumonia was 2,475 per 100,000, and the incidence of upper respiratory infection was 21,558 per 100,000 [7].

The Department of Quetzaltenango includes an area of 2,132 km² and has a population of 789,358 (2011), of which 41% are indigenous [5]. Located west of Guatemala City, the department comprises 24 *municipios*[1] (Figure 1 and 2). The department has three hospitals, two of which are located in the capital, Quetzaltenango (Xela), 21 health centers, and 66 health posts. The age distribution is as follows:

Age group	**Percentage of total population**
0-4 years	15%
5-9 years	14%
10-14 years	12%

[1] *Municipio* in Guatemala is similar to a county in the US.

15-19 years	11%
20-29 years	18%
30-39 years	11%
40-49 years	7%
50-59 years	5%
60 years +	7%

Quetzaltenango (also known as Xela) is the second largest city in Guatemala. Located at an altitude of 2,333 meters (7,655 feet) above sea level. . The city of Quetzaltenango is both the capital of the Department of Quetzaltenango and the municipal seat of the *municipio* of Quetzaltenango and has a large regional hospital with over 400 beds.

The incidence of diarrheal diseases in Quetzaltenango in 2012 was 2,851 per 100,000 residents [6]; the incidence of pneumonia and bronchopneumonia was 757 per 100,000, and the incidence of upper respiratory infection was 14,622 per 100,000 [7].

Surveillance sites

Surveillance will be conducted at the national hospitals of the selected departments, and at the health center of Nueva Santa Rosa and a health post in Xecam, Cantel, Quetzaltenango.

The National Hospital of Cuilapa is a 174-bed regional referral hospital located in Cuilapa, the capital city of the Department of Santa Rosa. The National Hospital of Cuilapa provides care for Santa Rosa residents and serves as a referral hospital for the neighboring municipalities of Jutiapa and Jalapa. It has 2 surgical wards, an emergency room, a laboratory and an outpatient department. It is a public, teaching hospital and, as part of the government's public health care system, does not charge for any services or medicines provided.

The Health Center of Nueva Santa Rosa is a type A health center with 24-hour care for emergency cases and a four-bed labor and delivery ward, although current construction of a new facility will expand their bed space. During the day, the clinic is staffed by 1 full-time physician, 3 auxiliary nurses and 1 professional nurse. Night shifts are shared by 3 private physicians and 3 nurses contracted by the MSPAS. It is equipped with electricity and a small laboratory staffed by a lab technician.

The Hospital of the Western Region in Quetzaltenango is a referral and teaching hospital with more than 400 beds. This hospital receives patients from Huehuetenango, San Marcos, Totonicapan, other departments in Guatemala, and Mexico. The hospital has 9 pediatric mechanical ventilators and 7 adult ventilators.

The Health Post in Xecam is a smaller facility that is not staffed by a physician. A nurse provides basic primary care to the surrounding population.

Case definitions, inclusion and exclusion criteria

NOTE: This section is also presented in Appendix A in table format for easier comprehension.

A. *Diarrhea* (surveillance to be conducted at all sites)

A.1. Case definition:

1. **Diarrhea**: The acute onset of 3 or more loose or liquid stools in a person in a 24-hour period during the last 14 days in a child < 5 years of age.
2. **Severe diarrhea**:
   Diarrhea as above with any one of the following:
   a. Lethargy or unconsciousness
   b. Sunken eyes
   c. Requiring intravenous fluids
   d. Presence of blood
   e. Vesikari score ≥11

A.2. Inclusion criteria

1. **Hospital**: Residents of the project catchment area during the last 30 days before presenting to the hospital admitted with a diagnosis of diarrhea;
2. **Health Center**: Residents of the selected *municipios* during the last 30 days before presenting to the health center with complaint of diarrhea;

3. **Health Posts**: Residents of the selected *municipios* during the last 30 days before presenting to the health post with complaint of diarrhea.

A.3. Exclusion criteria

1. Another episode of diarrhea in the 7 days before the start of this episode

B. *Respiratory disease*

B.1. Case definition:

1. **Pneumonia:** A person is considered to have pneumonia if they are admitted to the hospital and they have either 1.1.or 1.2

1.1: IEIP pneumonia case definition

a. Evidence of acute infection during the first 24 hours of admission (at least one of the following):

- Fever ≥ 38°C or a subjective history of fever
- Temperature <35.5°C with chills
- Abnormal white blood cell count (> 11,000/cm³ or <3,000/cm³) **or** abnormal white blood cell differential

AND

b. Signs or symptoms of respiratory tract disease (at least one sign or one symptom):

- Abnormal lung exam
- Tachypnea (defined as):
  age <2 months: ≥ 60/minute
  age 2—12 months: ≥ 50/minute
  age >12 months—5 years: ≥ 40/minute
  age >5 years: ≥ 20/minute

- Cough, sputum production, hemoptysis, chest pain **or** dyspnea, shortness of breath, sore throat, or,
- For children less than 2 years old: refusing to eat, drink, or breastfeed, repeatedly pausing to breathe while breastfeeding or drinking, nasal flaring, chest indrawing, or grunting.

1.2 Pneumonia case definition from WHO Integrated Management of Child Illness program for children <59 months of age

a. Age <2 months with tachypnea or chest indrawing

OR

b. Age <2 months with cough or difficulty breathing and at least one of the following:

- Cyanosis
- Not drinking or breastfeeding

- Vomiting all intake
- Convulsions
- Lethargy or fainting
- Head nodding
- Stridor at rest
- Hypoxia (O_2 saturation <90%)
- No movement or only when stimulated

OR

c) Age 2 to 59 months with cough or difficulty breathing and at least one of the following:

- Taquipnea
- Chest indrawing
- Cyanosis
- Not drinking or breastfeeding
- Vomiting all intake
- Convulsions
- Lethargy or fainting
- Head nodding
- Stridor at rest
- Hypoxemia (O_2 saturation <90%)

2. Influenza-Like Illness (ILI): A person is considered to have ILI if they are seen at the health center, health post, or private physician, and have:
a. A documented acute fever of ≥38^o C **AND**
b. History of cough **or** sore throat within the previous 14 days

B.2. Inclusion criteria

Same as for diarrhea

B.3. Exclusion criteria

There are no exclusion criteria.

C. *Acute febrile illness* (surveillance to be conducted at the hospital and health center)

C.1. Case definitions

1. **Hospital:**
   a. Self-reported fever during the current illness that began less than 7 days ago OR documented fever of ≥38ºC at presentation to hospital or within 24 hours of admission
2. **Health center**:
   a. Self-reported fever during the current illness that began less than 7 days ago OR documented fever of ≥38ºC at presentation to medical care

C.2. Inclusion criteria

1. **Hospital**:
   a. Resident of the project catchment area
2. **Health center**:
   a. Resident of the participating *municipios*

C.3. Exclusion criteria

1. **Hospital**:
   a. Evidence of an obvious source of fever on physical examination (eg. otitis media, septic arthritis, pyogenic soft tissue infection) determined by the examining healthcare provider and documented on the suspect case list.
2. **Health center**:
   a. Evidence of an obvious source of fever on physical examination (e.g., otitis media, septic arthritis, pyogenic soft tissue infection) determined by the examining healthcare provider and documented on the suspect case list;

Estimated number of participants

Diarrhea

Hospital: The National Hospital of Cuilapa reported 192 cases of diarrhea (19% under 5 years of age) in the first 6 months of 2006. We therefore expect to enroll approximately 384 cases per year. We expect to enroll approximately 700 cases of diarrhea per year in the Regional Hospital of Quetzaltenango.

Health center/health post: The health district of Nueva Santa Rosa reported 1122 cases of diarrhea (65% under 5 years of age) in the first 24 weeks of 2006. We estimate that 20% of these cases come from residents outside the surveillance area. Therefore, we expect to enroll approximately 2026 cases of diarrhea per year. We expect to enroll approximately 1000 cases of diarrhea per year in Quetzaltenango.

Pneumonia

Hospital: The National Hospital of Cuilapa reported 107 hospitalized cases of pneumonia in the first 6 months of 2006 using a loose case definition (37% under 5 years of age), and 49 cases using a more strict definition (47% under 5). Therefore, we expect to enroll between approximately 98 and 214 cases per year. We expect to enroll approximately 800 cases of pneumonia per year in the Regional Hospital of Quetzaltenango.

Influenza-like illness

Health center/health post: The health district of Nueva Santa Rosa reported 5104 cases (47% under 5 years of age) of acute respiratory infection in the first 24 weeks of 2006. We estimate that 20% of these cases are from outside the *municipio* and that 38% of them will be seen at the health center. We therefore expect to enroll approximately 2580 cases per year. We expect approximately 20 cases per week in the Santa Rosa health posts. We expect to enroll approximately 300 cases of influenza-like illness per year in Quetzaltenango.

Acute febrile illness

Hospital: Approximately 100 patients expected per year. We do not yet have plans to enroll cases of acute febrile illness in Quetzaltenango.

Health center: Approximately 500 patients expected per year. We do not yet have plans to enroll cases of acute febrile illness in Quetzaltenango

Recruitment/Enrollment

Inpatients: Subjects will be enrolled from the inpatient adult and pediatric medicine departments in the participating hospitals. Surveillance nurses identify potential cases in the emergency room and by reviewing the inpatient registers on each service using a list of suspect diagnoses and symptoms related to the disease syndromes included in this proposal. All patients who have at least one of the suspect diagnoses will be entered in the Suspect Case Form (H1) in the personal digital assistant (PDA). If they fit the inclusion/exclusion criteria, the nurse will request a verbal consent to ask screening questions. In the Selection Form (H2), the questions determine whether or not the patient satisfies the case definition. If the patient is eligible to participate, the nurse will request written, informed consent and assent, if required, and will then continue with the questionnaire and sample taking.

Outpatients: Subjects will be enrolled at the participating Health Center and Health Post. When a potential case is identified by one of the surveillance nurses, she will request a verbal consent and complete the selection form to determine whether the case definition has been met. If the patient meets one of the case definitions and satisfies the inclusion/exclusion criteria, the nurse will obtain written consent and or assent, if required, from the patient and/or family member.

Upon entering the study, a unique identifier will be assigned to each patient, consisting of a series of numbers with an alphabetic identification code using the first 2 letters of the first name and the last 2 letters of the last name of the subject. This unique identifier will be written on all study forms and specimens to ensure information remains confidential and avoid confusion.

Follow-up

We will ask all hospital, health center and health post subjects if we may contact them by phone after study entry to complete study information, including vital status, or invite them to participate in future studies. The determination of whether to call a participant will be made by the principal linvestigator

Consent process

Inpatients: The project surveillance nurse will seek consent from eligible patients and/or their responsible family members. If the patient is under 18 years of age or unable to provide consent due to altered mental status, the nurse will request permission to participate in the project from the patient's family member or guardian. If the patient is 7 - 17 years of age, assent will be obtained from the patient if the parent or guardian has provided consent.

The attending physician will decide whether or not the patient is capable to give the informed consent. In cases where a patient is initially not capable of giving consent, consent will first be obtained from family members. Once the patient regains mental competence to give consent, he or she will be contacted to allow the patient the opportunity to refuse further involvement in the project and to ask to discard data and residual specimens.

Outpatients: The project nurse will seek consent from eligible patients and/or their responsible family members. If the patient is under 18 years of age, the nurse will request permission to participate in the project from the patient's family member or guardian. If the patient is 7 - 17 years of age, assent will be obtained from the patient if the parent or guardian has provided consent. Group consent, where the form is read to several eligible patients at one time although each signs individually, may be used depending on the clinic patient flow.

Confidentiality

Participants' names and age will be recorded in order to link their clinical and laboratory data for reports to the MSPAS. At enrollment, subjects will be assigned a unique project identification number that will be used to label all project materials and specimens for transport to the labs at CES/UVG-CDC/CAP and storage. Completed case report forms and laboratory information will be kept in a locked file cabinet in a locked office at CES/UVG, accessible only to project personnel. All data files will be maintained as password protected files on password protected computers. The project physicians, project surveillance nurses and principal investigators will have access to personal identifiers for the purpose of follow-up. At the end of the project the PI will remove all identifying information and use anonymous codes to link patient information between databases. We anticipate preparing one or more manuscripts for publication.

Demographic, clinical and risk factor data collection

Inpatients: Data collected will include basic demographic, clinical (symptoms, signs, treatment prior to enrollment), and risk factor (exposures 1 month before the onset, travel history, significant medical history) information. In addition, a discharge form will include relevant laboratory analyses (e.g. CBC, chemistry, blood culture, HIV status). Chest radiographic studies that have been performed as part of the subject's standard clinical care at the hospital will be digitized and sent to a panel of radiologists in Guatemala for review. Data on type and duration of treatment and duration of hospitalization will also be collected to document the clinical course of subject at the hospital. The subject's condition on discharge from the hospital and outcome (death versus discharge) will be noted.

Surveillance data will be collected using hand-held, password protected, personal digital assistants (PDAs) using information from the subject's chart and from the subject's responses to the questionnaire. If the patient's blood culture result is not in the chart (as is often the case), the nurse is authorized to look up the result in the hospital laboratory's blood culture book. If the result is still not found in the blood culture book, the study IT staff may later obtain the blood culture result from the hospital's existing blood culture database.

Outpatients: Data collected will include basic demographic, clinical (symptoms, signs, treatment prior to enrollment), and epidemiological (exposures 1 month before the onset, travel history, significant medical history) information.

Sample collection

A. *Diarrhea*

Stool: A whole stool sample of ≥ 5 g will be requested from all consenting subjects < 5 years of age enrolled with diarrhea. If a subject is not able to produce a whole stool sample the following algorithm will be followed based on the level of health care the patient visited:

- Hospitalized patient: the project nurse will collect a rectal swab from children with consent of the parent/guardian. Secondary attempts to collect a whole stool sample will be made by the project nurse during the first three days and preferably first 24 hours after admission. If a fecal specimen has already been collected for another study, that specimen will be used, so as not to further inconvenience the subject.
- Health center and/or health post patients: a rectal swab will be obtained from the child of a consenting caretaker; the subject will be asked to return within next three days and preferably within 24 hours after the visit with a whole stool sample.

Samples from the health posts and health center will be stored in an insulated cooler at 4°C until transported to the participating hospitals. At the hospital, all stool samples will be aliquotted into appropriate media (formalin)) or kept fresh and at the appropriate temperature for weekly transport to the laboratories at the UVG.

B. ***Pneumonia***

Nasopharyngeal swabs: Within the first 24 hours of admission, one Dacron nasopharyngeal swab will be placed into one nostril to the nasopharynx and rotated once to collect epithelial tissue and absorb secretions. The swab will be placed along with the oropharyngeal swab into 3% nutrient viral transport broth for viral isolation. It will be stored in a refrigerator for up to 48 hours until transported to storage at the hospital or UVG lab at -70°C.

Oropharyngeal swab: One oropharyngeal swab will be collected from all consenting patients in the first 24 hours. The sample will be processed together with the nasopharyngeal swab.

Urine: 20 ml of urine will be collected in the first 24 hours from each consenting patient aged ≥18 years and refrigerated at 4°C until transport to the UVG. A 2 ml aliquot will be preserved at -70°C.

C. ***Influenza-like illness***

Nasopharyngeal swabs: One Dacron nasopharyngeal swab will be placed into one nostril to the nasopharynx and rotated once to collect epithelial tissue and absorb secretions. The swab will be placed into 3% nutrient viral transport broth for viral isolation. It will be stored in a refrigerator for up to 48 hours at 2-8°C until transported to storage at the hospital or UVG at -70°C.

D. ***Acute febrile illness***

Blood: The national hospitals have as part of their protocol the taking of blood samples for culture, CBCs and chemistry if there are indications of pneumonia and septicemia. These samples are taken once a decision has been made to admit the patient. Generally, a portion of the sample is left over from that used for chemistry. We will try to use this additional sample to perform acute-phase PCR and serological testing. However, if no blood was taken on admission, we will request 5 ml from children ≤12 years and 10 ml from patients >12 years old using sterile techniques to obtain an acute blood sample. 4mL will be drawn into a vacutainer tube with EDTA, refrigerated at 4°C and transported to UVG. After serum is separated, 3 equal aliquots of 0.5 ml each will be frozen at -70°C. One serum aliquot will remain at UVG,and one will be sent to the laboratory in CDC for confirmatory testing.

Ticks, lice, fleas: Any ticks, lice, or fleas recoverable from the patient at presentation will be collected in a cryovial, and kept frozen at -20 ºC until shipment on dry ice to the Rickettsial laboratory in Atlanta for testing.

Sample analysis and laboratory diagnostics

Specimens will be tested using Binax, culture, PCR, antigen tests and antibody tests at the hospital, at the laboratories of the Universidad del Valle de Guatemala in Guatemala City and at CDC in Atlanta and Fort Collins.

A. Diarrheal illness samples:

- Stool samples from the ambulatory sites will be stored in Cary-Blair media at 4°C, and transported in temperature-monitored containers (4°C) within 24 hours of collection, to one of the two regional hospital laboratories for initial analysis.
- Samples will be tested for presence of parasites (helmints and protozoa, for complete list see Appendix "C") by direct smear examination[8], bacteria (*Salmonella, Shigella, Campylobacter*) by direct culture[9], and rotavirus (by using a commercial qualitative enzyme immunoassay (IDEIA Rotavirus test kits, Oxoid Ltd., Ely, United Kingdom)[10]. Testing for Norovirus (genogroups I and II) using a standard monoplex qRT-PCR [11] and quality control assessments will be performed at the laboratory at the Universidad del Valle de Guatemala.. Pathogenic strains of E.coli will be analyzed by conventional PCR techniques 15,16.
- Further molecular tests will be carried out at the CDC laboratories in Atlanta.

B. Respiratory illness samples:

- Respiratory samples (OP and NP swabs) will be processed by qRT-PCR to detect RSV, hMPV, Parainflueza, adenovirus and influenza virus. [12, 13
- Selected respiratory specimens from adults will also be tested using the Taqman Array Card (TAC) (14) The TAC protocol has been previously approved by the CDC and UVG IRB's and consist of a case control study in which the cases will be severe acute respiratory infections in persons aged ≥18 that are captured by VICO and for whichthe same specimen, VICO informed consent form and VICO questionnaire will be used.
- Chest x-rays will be digitized and stored by the unique number of the patient and will be reviewed on an ongoing basis by a panel of radiologists to determine whether the patient has clinical pneumonia. Each x-ray will be read by two radiologists with any differences being resolved by a third radiologist.

C. **Acute febrile illness samples: Febrile samples (blood and serum) will be tested by PCR to detect dengue, rickettsia and bartonella and by ELISA IgM to detect dengue and leptospira.**

As mentioned previously, all samples will be labeled with a unique ID number that will also be used on all forms to ensure that patient information and diagnostic results are linked..

Specimen storage

Remaining specimens from patients who have agreed to participate in the study and who have agreed in writing to have their samples stored will be warehoused at the UVG and Caspir storage facilities in Atlanta for possible future tests. Future diagnostics could be undertaken if there are new diagnostics available or a new pathogen to be detected. Specimens of patients who are not in agreement to have their specimen stored or have revoked their consent will be autoclaved at 121^{o}C for 30 minutes and thereby destroyed.

Routine audit activities

The Project Supervisor will visit the hospital weekly and the other facilities at least once per month to verify that project procedures are being followed correctly and that subjects are being recruited according to the protocol.

Data management and analysis

Subjects will be issued a unique identification number (DASH number) that will be used on all study forms, specimens and chest x-rays. This code will be supplemented by an alphabetic code formed from the first three letters of the first and last name of the patient to ensure that the link between the data and specimens is maintained. Consent forms will be prepared on Teleforms (Cardiff Software) and scanned for data entry after visual inspection by the Project Supervisor. All other study information will be collected using a software developed in-house, Questionnaire Mobile, installed on personal digital assistants (PDAs) which will be used by project nurses. Data will be backed up daily to central servers at Universidad del Valle de Guatemala, and databases will be stored in password-secured servers.

Cleaning and data analysis will be performed using appropriate statistical software (SAS, R, or equivalents). The "Objectives" section describes specific aims for results from surveillance.

Adverse events

Inpatients: All samples are standard medical procedures for diagnostic purposes and will be collected under the supervision of hospital personnel contracted to support this project; any adverse events associated with these procedures will be dealt with by hospital doctors and administrative staff according to standard procedures.

Outpatients: We do not anticipate any adverse events associated with provision of stool samples for patients with diarrhea. There is the possibility that the use of nasopharyngeal swabs for samples from cases of ILI may cause bleeding of the nose. All blood samples for acute febrile illness (venipuncture) will be collected under the supervision of the study nurse contracted to support this project; any adverse events associated with these procedures will be dealt with by the study nurse according to standard procedures.

Dissemination and reporting of results

For the majority of cases, results of etiological testing performed at UVG/CES and CDC will not be available for clinical management because of the expected delays required for storage,

shipment and conducting confirmatory tests. All confirmed etiologies will be provided regularly to the Health Area, the participating hospital and clinics and the MSPAS. We will provide these data to each respective clinic, and the clinic will report positive results to the patients if indicated. Reporting these results directly to the individual patient will not be the responsibility of the project investigators. If a particular patient's results have clinical or public health relevance, the patient will be contacted. This information will allow the Public Health Ministry to take appropriate action to protect the health of the patient and the community.

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Appendix A

Summary of Project Procedures by Disease Syndrome and Location

Diarrhea

	Hospital Inpatient	**Health Center**	**Health Post**
Case definition:	≥3 loose or liquid stools in 24 hours in last 14 days		
Inclusion criteria:	Residents of project catchment area admission diagnosis of diarrhea Age < 5 years	Residents of participating municipios, complaint of diarrhea Age < 5 years	Residents of participating municipios, complaint of diarrhea Age < 5 years
Exclusion criteria:	Another episode of diarrhea in the 7 days before the start of this episode		
Samples to be taken:	≥5 grams stool or rectal swab		

Respiratory Disease

	Hospital Inpatient	Health Center and Health Post
Case definition:	**IEIP Pneumonia** 1. Evidence of acute infection during the first 24 hours of admission (at least one of the following): • Fever ≥ 38⁰ C or subjective history of fever • Temperature <35.5⁰C with chills • Abnormal white blood cell count (> 11,000/cm³ or <3,000/cm³) **or** abnormal white blood cell differential **AND** 2. Signs or symptoms of respiratory tract disease (at least one of the following): : •Abnormal lung exam •Tachypnea, : •Cough, sputum production, hemoptysis, chest pain, dyspnea, shortness of breath, sore throat, •For children <2 years old, doesn't want to eat, drink or breastfeed, repeatedly pausing to breathe while breastfeeding or drinking, nasal flaring, chest indrawing, or grunting. **IMCI Pneumonia** Pneumonia case definition from WHO Integrated Management of Child Illness program for children <59 months of age 1. Age <2 months with tachypnea or chest indrawing OR 2. Age <2 months with cough or difficulty breathing and at least one of the following: • Cyanosis • Not drinking or breastfeeding • Vomiting all intake • Convulsions • Lethargy or fainting • Head nodding • Stridor at rest • Hypoxia (O_2 saturation <90%)	ILI • Documented fever (≥38C) AND • Cough or sore throat within last 14 days

	Hospital Inpatient	Health Center and Health Post
Inclusion criteria:	Residents of project catchment area	Residents of project catchment area
Exclusion criteria:	None	None
Samples to be taken at admission:	• Nasopharyngeal swab • Oropharyngeal swab • 20 ml of urine for patients older than 18 years	Nasopharyngeal swab.

Acute Febrile Illness

	Hospital Inpatient	Health Center	
Case definition:	Self-reported fever during the current illness that began <7 days ago OR documented fever of ≥38ºC at presentation to hospital or within 24 hours of admission	Self-reported fever during the current illness that began <7 days ago OR documented fever of ≥38ºC at presentation to health center	
Inclusion criteria:	Resident of Santa Rosa, Jutiapa, o Jalapa	Resident of the municipio	
Exclusion criteria:	• Evidence of an obvious source of fever on physical examination	• Evidence of an obvious source of fever on physical examination	
Sample to be taken on presentation:	(If no extra sample) Blood: 12.5-15 ml from children ≤ 12 years of age and 17.5-20 ml from >12 years	Blood: 5 ml from children ≤12 years and 10 ml from >12 years.	Blood: 4 ml from adults and children

Appendix B

Guide to data collection forms

Hospital

Form type	Diarrhea	Respiratory Disease	Acute Febrile Illness
Identification of suspect cases	H1 Suspect Case Log		
Enrollment/Application of case definition	H2 Enrollment form		
Consent	Adult, Adult Unable, Adult Formerly Unable, Child		
Assent	Child Assent		
Case report	H3 A-K		
Patient update form	HCD4 (Diarrhea), HCR4 (Respiratory)		
Samples	H5		
Sample transport log	L001		
Radiology		HR6 CXR Results	
Discharge form	H7		
Diagnostic laboratory results	L002	L003	L004 / L005

Termination form	HCP11

*Forms highlighted in yellow are used in multiple project sites (hospital plus health center and health post).

Health Center and Health Posts

Form type	Diarrhea	ILI**	Acute Febrile Illness
Suspect case log and enrollment	C1 and P1		
Consent	Adult, Child		
Assent	Child Assent		
Case report	C2 and P2		
Patient update form and follow-up	HC4		HC4
Samples	C5 and P5		
Visit results	C7 and P7		
Sample transport log	L001		
Diagnostic laboratory results	L002	L003	L004
Follow-up	HC9		HC9
Termination form	HCP11		

*Forms highlighted in yellow are used in multiple project sites (hospital plus health center and health post).

APPENDIX C: List of pathogens samples will be evaluated for and corresponding test

A. Diarrheal diseases

Category	Specific	Test performed/Method employed
Bacteria	Salmonella spp. Shigella spp. Campylobacter sp. Escherichia coli (ETEC, EPEC)	Culture Conventional PCR
Virus	Rotavirus Norovirus 1 and 2 Sapovirus Astrovirus	ELISA IgM RT-PCR RT-PCR RT-PCR
Parasites	*Ascaris lumbricoides* *Trichiuris trichiura* *Hyminolepis nana* *Hyminolepis diminuta* *Uncinaria* *Enterobius vermicularis* *Iodamoeba butshchlii* *Endolimax nana* *Chilomastix mesnilli* *Blastocystis hominis* *Entamoeba coli* *Giardia lamblia* *Cryptosporidium parvum*	Micrsocopy (fresh smear)

B. Respiratory diseases

Category	Specific	Test performed/Method employed
Bacteria	*Mycoplasma pneumonie* *Chlamydia pneumonie* Legionella sp	RT-PCR
Virus	RSV hMPV Parainfluenza 1, 2 ,3 Adenovirus Influeza A (subtyping) and B	RT-PCR

C. Acute febrile illness

Category	Specific	Test performed/Method employed
Bacteria	Rickettsia Bartonella Leptospira	Conventional PCR Conventional PCR ELISA IgM
Virus	Dengue	RT-PCR and ELISA IgM
Parasites	Malaria	Thick smear