

Annexes

8.1. Annex I – Surveillance of Hemolytic Syndrome (Attached A)

I - Introduction

Escherichia coli O157:H7 causes diarrhea (usually bloody, and called hemorrhagic colitis) and abdominal cramps with little or no fever. Approximately 10% of cases develop hemolytic uremic syndrome (HUS), which is characterized by acute renal injury, thrombocytopenia and microangiopathic anemia. Other Shiga-toxin-producing *E. coli* (STEC) also cause diarrhea and HUS; production of Shiga toxin is thought to be essential for development of this classical form of HUS. Although HUS can occur in persons of any age, previously healthy children are most often affected. The main clinical manifestations are renal insufficiency (40% of patients present oliguria or anuria), pallor, and petechiae or bruises. Acute neurological complications such as lethargy, stroke, seizure, and coma develop in 25% of patients.

In the 1950s and early 1960s the mortality rate for HUS was >30%. After that, with the institution of early dialysis for severe oliguria or anuria, mortality rate decreased, and is now 2-3%. However, 5% of children with HUS develop chronic renal insufficiency and in a few years develop end-stage renal disease which require dialysis or kidney transplantation. Another 30% of the patients have microhematuria or chronic proteinuria; some of these develop end-stage renal failure, years or even decades later.

Human infection with *E. coli* O157 and other STEC has been reported from over 30 countries on six continents. High rates are present in Argentina, where HUS is endemic. In Argentina, ~250 new cases of HUS are reported annually by Hospital Nephrology Units to the Argentine Society of Pediatrics. At present, the estimated annual incidence rate for HUS is 7.8 per 100,000 in children under five years of age. Over 6,000 cases of HUS have been reported since 1965. Children affected are usually under five years, mostly between 6 and 36 months. Both sexes are equally affected and cases tend to come from middle income socioeconomic groups, who are well nourished and living in good sanitary domestic conditions. In these children, the prodromal diarrhea generally is often first diarrheal episode in their lives. The illness is distributed throughout the whole country, but cases are reported more frequently in central and southern states during warmer months.

Recent studies have supported the idea that all virtually cases of postdiarrheal HUS in developed areas have gastrointestinal infection by *E. coli* O157:H7 or other STEC in the 2 weeks before onset of the acute renal failure. (The only other known cause of postdiarrheal HUS is *Shigella dysenteriae* type 1, the only *Shigella* species that produces Shiga toxin.) In the United States, the risk of HUS after *E. coli* O157 infection is ~5% in the outbreak setting where mild cases are detected, and 10-15% in children who present with bloody diarrhea for medical care. The proportion of children who develop HUS after infection with other STEC

is not known. In Argentina, HUS affects infants and children younger than in the northern hemisphere and it is possible that the risk for developing HUS after STEC infection is greater.

Healthy cattle are a major reservoir of STEC and they are transmitted principally through consumption of contaminated foods, such as raw or undercooked ground meat and raw milk. Fecal contamination of water and other foods and cross-contamination during food preparation have been important routes of infection. Examples of food implicated in outbreaks of *E. coli* O157 infection include hamburgers, roast beef, raw milk, fresh-pressed apple juice, yogurt, cheese, fermented sausage, cooked maize, mayonnaise, lettuce and seed sprouts. The pathogen is relatively acid tolerant and can survive in fermented foods and fresh vegetable produce. Other animals, principally sheep and deer, also carry STEC strains that are pathogenic for humans.

In Argentina, in a study with 34 HUS patients and 95 household contacts (20 children and 75 adults) it was demonstrated that in 24% of HUS patients, 1 to 6 family members presented diarrhea simultaneously or in the 7 previous days. From the 95 household contacts, 31,6% showed one or more diagnostic criteria for STEC infection (specific free fecal Shiga toxin, STEC isolation and/or Stx-neutralizing antibodies). So, person to person transmission seems to be an important way to acquire the disease.

HUS is a serious illness whose diagnosis does not depend on laboratory confirmation. The incidence of HUS may be used as a robust and consistent marker for STEC infections. Surveillance for HUS is recommended in order to provide a relatively stable baseline for comparisons over time. Countries should be consider the implementation of a nationwide mandatory notification system or HUS.

Overview of proposed surveillance systems and studies. We propose the following surveillance systems and studies:

1. Basic surveillance for childhood postdiarrheal HUS in all southern cone countries.
2. Active hospital-based sentinel site surveillance for childhood postdiarrheal HUS in countries which choose this method for improved case ascertainment.
3. Laboratory investigation of childhood postdiarrheal HUS cases in hospital-based sentinel sites in countries with interest in determining the STEC serotypes that cause HUS in their country.
4. Hospital laboratory-based sentinel site study to investigate the major causes of bloody diarrhea in children (Attachment A).
5. Case-control study of children with STEC infection in sentinel sites to determine risk factors for infection (e.g. food, water, person-to-person transmission, exposure to farms and animals, etc). (Protocol not yet written, but budget provided).

Case definition for childhood HUS for surveillance

CLINICAL DESCRIPTION

Hemolytic uremic syndrome (HUS) is characterized by the acute onset of microangiopathic hemolytic anemia, renal injury, and low platelet count following an acute episode of bloody

or nonbloody diarrhea in a previously healthy child. Pallor, oliguria, and petechiae or bruises are common manifestations; neurological symptoms such as lethargy or seizures may also be present.

LABORATORY CRITERIA FOR DIAGNOSIS

The following are both present at some time during the illness:

- Anemia (acute onset, with Hb <10 mg/dL, or Hct <30%, or a fall in Hct of >5%) with microangiopathic changes (i.e. schistocytes, burr cells, or helmet cells) on peripheral blood smear, and
- Renal injury (acute onset) evidenced by either hematuria, proteinuria, blood urea >50mg/dl in the absence of dehydration, or creatinine >1.0 mg/dL (in a child) or \geq 50% increase over baseline

Note: If a platelet count obtained within 7 days after onset of the acute gastrointestinal illness is not <150,000/mm, other diagnoses should be considered.

Case Classification

PROBABLE CHILDHOOD CASE

An acute illness diagnosed as HUS in a hospitalized child that meets the laboratory criteria in a patient who does not have a clear history of an acute episode of bloody or nonbloody diarrhea in the preceding 3 weeks; or

An acute illness diagnosed as HUS in a hospitalized child that has onset within 3 weeks after onset of an acute episode of bloody or nonbloody diarrhea and meet the laboratory criteria except that microangiopathic changes are not confirmed.

CONFIRMED CHILDHOOD CASE

An acute illness diagnosed as HUS in a hospitalized child that meets the laboratory criteria and began within 3 weeks after onset of an acute episode of bloody or nonbloody diarrhea.

Surveillance for HUS

We recommend that southern cone countries make postdiarrheal HUS reportable. All pediatricians (especially pediatric nephrologists) and infection control personnel in pediatric hospitals should be advised to report hospitalized cases according to a schedule and method decided by the national health department. In some locations, reporting may be facilitated by collaborating with the pediatric nephrology society. In countries where hospitals report cases of notifiable diseases weekly, HUS should be included in this report. Minimum information

obtained from each case should include date of hospital admission, date of birth or age, sex, code for residence in the 21 days before onset of HUS, and whether child survived or died. The national health department will tabulate cases at least annually.

SYSTEMS FOR ACTIVE SURVEILLANCE

1. Hospital-based system for identification of HUS cases;
2. Hospital-based system for identification of HUS cases with local laboratory collaboration in testing stool and serum for STEC infection in sentinel sites.

Countries with few or no recognized HUS cases should consider sentinel-site hospital-based surveillance. Criteria for sentinel-site selection: a) interest and availability of a local coordinator (e.g. doctor or nurse from the hospital, person from the local health department b) interest and availability of laboratory personnel at the hospital (for surveillance systems that include laboratory testing for STEC) c) presence of cases of HUS in the area in the past; d) likelihood of cases being admitted to this hospital; e) selection of hospitals from different geographic areas.

SURVEILLANCE OF CHILDHOOD HUS WILL ALLOW THE FOLLOWING QUESTIONS TO BE ADDRESSED

1. What is the incidence and mortality in the southern cone countries?
2. Which age groups are at risk to develop HUS?
3. Are both sexes equally affected?
4. Are cases from rural or urban areas?
5. What are trends in number of cases over time, by age and location?

In addition, because only ~10% of children with STEC infection develop HUS, detection of a cluster of cases of HUS should alert the health department to other cases of STEC infection, and the source of the illnesses can be investigated.

Laboratory evaluation of stool and sera samples from patients with HUS will allow the following questions to be addressed:

1. What proportion of cases of HUS in the southern cone countries are associated with *E. coli* O157 or non-O157 STEC infection? Is there any geographic variation?
2. Which O:H serotypes are associated with HUS?
3. Which is the Shiga toxin prevalent in the STEC isolates?
4. Which other virulence factors are present in the STEC isolates?
5. What is the genetic relatedness between the isolates?

LABORATORY CONFIRMATION OF STEC IS DEFINED AS

- a) Isolation from stools of *E. coli* O157:H7, Shiga toxin-producing *E. coli* O157:NM, or other STEC,
- b) An acute serological response to one of these known pathogens

II. Protocol Design

We propose to study all cases of HUS admitted to selected major medical centers. The announcement of the study and request for participation from interested hospitals will be done in a hospital newsletter with national circulation. Centers will be selected for participation based on willingness to participate, the geographic location and laboratory availability. An investigator at each medical center will be contacted, this local investigator will usually be a nephrologist, an infectious disease physician or a hematologist.

The study coordinator in each center will be responsible for notifying the appropriate clinicians (e.g. pediatric nephrologist and others) prospectively about the study. He should encourage early collection of stool specimens from patients with possible HUS since collection of stools specimens early in the illness may be critical in making a diagnosis of STEC infection. When a patient with possible HUS is admitted to the medical center, he will be responsible for all aspects of that patient's involvement in the study. He will arrange for consent/assent to be requested. He will insure that stool and blood samples are obtained promptly and handled appropriately. He will make arrangements for a convalescent serum to be drawn at no expense to the patient. He will insure that the questionnaire is completed and returned in a timely fashion. If the results of this investigation are published, the local coordinator and the microbiologist who have made a significant contribution will be authors.

LABORATORY

Stool specimens will be visually inspected for gross blood and the presence of occult blood. The presence of fecal leukocytes will be determined by placing a bit of stool in a drop of methylene blue or by doing a Gram stain and examining the specimen using the high-power microscope objective. Specimens will be graded as having 0, 1- 4, 5 - 9 or 10 or more leukocytes per high-power field.

To identify *E. coli* O157:H7, all fecal specimens will be plated onto sorbitol-MacConkey agar and plates will be incubated at 37°C for 24 h. Three sorbitol-negative colonies will be tested for agglutination with O157 antiserum. The O157-positive colonies identified as *E. coli* will be sent to the National Reference Laboratory (NRL) to be tested for production of Shiga toxin (Stx) 1 and 2 and for the presence of *stx* genes by PCR and by hybridization with oligonucleotide probes. Other virulence factors such as *eaeA*, E-Hly and Stx2 variants will be investigated. Isolates will be tested by using the disk diffusion technique for susceptibility to a standard panel of antimicrobial agents.

To identify non-O157 *E. coli* three sorbitol-positive colonies will be tested for *Stx* genes by PCR at the NRL.

Acute and convalescent antisera will be tested for anti-O157 LPS antibodies by ELISA. Seroconversion will be defined as fourfold titer increase between the two samples.

III. Protocol Flow

1. When a patient with possible HUS is admitted to a participating center, consent for the study will be obtained. The parent or guardian will sign the “Child Form”.
2. The first stool obtained will be aliquoted into 2 samples: one sample will be submitted immediately to the hospital laboratory and the second one will be kept frozen at -70°C or submitted to National Reference Laboratory. If rectal swabs is used they should be placed in Cary Blair transport media and maintained in the same conditions as the stool samples. The second sample should be sent on dry ice to Dr.....of the National Reference Laboratory. Stool samples should be labeled “HUS Study” and come with the following information:
 - Patient’s full name
 - Date specimen obtained
 - Name and phone of notifying physician

Physicians are encouraged to obtain a stool specimen as soon as the diagnosis of HUS is considered, since previous works indicated that the rate of positive stool cultures for STEC decreases after the few days of illness

3. A serum sample (at least 1 ml) will be obtained at the time that the diagnosis of HUS is made, frozen at -20°C and sent to Dr.....of the National Reference Laboratory on dry ice with the stool sample. Serum sample should be labeled “HUS Study” and come with the following information:
 - Patient’s full name
 - Date specimen obtained
 - Name and phone of notifying physician
4. Part I of the questionnaire will be completed by the patient’s physician or by the local study coordinator within 48 hours after the diagnosis of possible HUS
5. Part II of the questionnaire will be completed by the patient’s physician or by the local study coordinator at the time of the patient’s discharge from the hospital using information in the patient’s chart. It will be sent to the Central Coordinator (NRL) at the address listed at the top of the form.
6. The convalescent serum sample will be obtained 3 to 4 weeks after the onset of symptoms and at least 2 weeks after the acute serum, frozen at -20°C and sent to Dr. _____ of the National Reference Laboratory on dry ice with the stool sample. Serum sample should be labeled “HUS Study” and come with the following information:
 - Patient’s full name
 - Date specimen obtained
 - Name and phone of notifying physician

7. All records obtained in this study will be kept by the Central Coordinator at the NRL. Each record will include the consent form, questionnaire and results of stool and serum studies.

IV. Flow Diagram for Study

1. Initial consultation with nephrologists at National Society of Pediatrics (it should be different for each country).
2. Contact made between NRL and medical center investigator with interest in HUS
3. The appointed local study coordinator at medical center notifies clinicians about the study and the importance of early stool sampling.
4. Patient with possible HUS admitted to hospital.
5. Consent obtained from parent.
6. Stool sample immediately sent to hospital laboratory and duplicate stool sample and acute serum sent to NRL
7. Part I of questionnaire completed.
8. Convalescent serum sent to NRL
9. Part II of questionnaire completed and sent with consent forms to NRL.
10. Preliminary test results sent to study coordinator at medical center and Central coordinator at NRL.

V. Budget for First Year

Budget for the meeting for training and organization of surveillance activities, September 1999, Buenos Aires;

Budget for attendance of 22 persons, including 10 persons from Argentina, 2 persons from each of Bolivia, Chile, Paraguay, Uruguay and 4 from Brazil. Persons dealing with organization and clinical aspects will meet for 5 days; this will be followed by a training course for laboratory personnel, so laboratory personnel will meet for a total of 14 days.

South American meeting attendees

Food and lodging	21, 280
Travel	<u>8,500</u>
<i>Subtotal</i>	<i>29,780</i>

Training course materials

Laboratory reagents	5,500
Paper, manuals	2,000
Secretarial assistance	1,000
Other supplies, e.g. telephone, audiovisual aids	<u>1,500</u>
Subtotal	<u>10,000</u>
Total (not including CDC costs)	<u>39, 780</u>

Costs for CDC collaborator attending above meeting and planning studies with local collaborators

Food and lodging x 2 weeks	1,500
Travel	<u>1,500</u>
<i>Total for CDC collaborator</i>	<i>3,000</i>

Basic surveillance for HUS (Annual cost for each country)

Design of surveillance form (1 st year only)	1,000
Data input and tabulation	3,000
Paper, supplies, telephone calls to hospitals	<u>500</u>
<i>Subtotal</i>	<i>4,500</i>

Laboratory reagents

Reagents (\$10,000/country x 5)	50,000
Additional reagents for 10 hospital labs in Argentina participating in lab study of HUS and case-control study	25,000
Additional reagents for LNR, Argentina	<u>20,000</u>
<i>Subtotal for reagents</i>	<i>95,000</i>

Argentine study personnel for laboratory study of HUS and case-control study

Ph.D. level microbiologist	14,400
Technician	8,400
Clerical assistance	8,400
Interviewers (1/hospital x 10)	<u>3,600</u>
<i>Subtotal</i>	<i>30,200</i>

Argentine laboratory equipment and supplies

Freezer, -70C	15,000
Freezer, -20C	1,000
Refrigerator	<u>1,000</u>
<i>Subtotal</i>	<i>17,000</i>

Shipping and handling of lab specimens

Shipping and handling (1,000/country x 5)	5,000
Additional cost for large number of samples in Argentina	5,000

Meeting to plan case-control study, December 1999, Buenos Aires

Personnel from Argentina

Food and lodging for 20 persons	2,800
Travel for 20 persons	4,200
Supplies	1,000
Clerical assistance	<u>2,000</u>

Subtotal 10,000

Collaborator from CDC, costs for planning case-control

Study food and lodging x 3 weeks	2,100
Travel	<u>1,500</u>

Subtotal 3,600

Meetings in Bolivia to initiate surveillance for HUS

Meeting with pediatricians to train them in recognizing and reporting HUS;
Meeting with microbiologists from pediatric hospitals to train them in identifying *E. coli* O157 (See Attachment B for description)

Travel for 9 persons	1,663
Lodging for Bolivian attendees (8 persons x 6 days)	840
Food	1,920
Supplies (paper, etc. for course)	300
Lodging for international attendees (1 person x 6 days)	420
Laboratory reagents for course	1,000

Subtotal 6,143

TOTAL South American costs 212,623

TOTAL CDC personnel costs 6,600

GRAND TOTAL **219,223**

Surveillance of E. coli 0157 H7 and Hemolytic Uremic Syndrome in Bolivia – Protocol for a First Meeting and Laboratory Course (Attached B)

INTRODUCTION

Taking into account the recommendations approved in Buenos Aires, 1998, and reiterated during the meeting in Brasilia, 1999, Bolivia has decided to become involved the surveillance of *E. coli* O157 H7 and Hemolytic Uremic Syndrome. For this purpose, INLASA's laboratory of diarrhea and cholera diseases will conduct this work in coordination with sentinel pediatrician hospitals (with microbiological laboratory) from three main cities of the country: La Paz (2), Cochabamba (1) and Santa Cruz (1).

Objectives

In order to initiate HUS surveillance a national meeting will be held with the attendance of pediatricians and microbiologist from de selected places. This meeting will be organized by Dr. Esther Damiani (INLASA), and Dr. Daisy Bocangel (Pediatician from the National Pediatric Hospital, La Paz) in coordination with the National Office of Epidemiology.

The purpose of this meeting is to developa uniform protocol for:

- 1) Surveillance of HUS
- 2) Microbiological research of *E. coli* O157 H7 in children with HUS, and/or in children below five years old with bloody diarrhea (this research will include *Campylobacter* and *Shigella*).

Methodology

- A three days meeting with all the participants (HUS surveillance).
- Five days course for microbiologists. For the course of microbiological research will be invited Dra. Marta Rivas from C. Malbrán Institute, Buenos Aires, Argentina.

Participants

2 Pediatricians from the selected hospitals (or 1 pediatician and 1 epidemiologist)	Total 6
2 Microbiologists from the selected hospitals	Total 6
Personnel from INLASA's Laboratory of EDAS/Cholera	Total 6

Funding: CDC, Atlanta, through PAHO/WHO, Bolivia

Organization: INLASA, La Paz, Bolivia

Place: Instituto Nacional de Laboratorios de Salud, INLASA, La Paz, Bolivia

Budget

Airfare:	
-1 Bs. As. – La Paz – Bs. As.	US\$ 523.-
-4 Santa Cruz – La Paz – Santa Cruz	US\$ 788.-
-4 Cbba – La Paz- Cbba.	US\$ 352.-
Accommodation in La Paz - Bolivia	
-8 persons x 6 days (nationals)	US\$ 840.-
-1 person x 6 days (international)	US\$ 420.-
Food	US\$ 1,920.-
Laboratory reagents for the course	US\$ 1,000.-
Supplies	US\$ 300.-

TOTAL workshop US\$ 6,143-