II. The Role of the Public Health Laboratory

A. General Considerations

An understanding of the use of laboratory results is essential in guiding the selection of any laboratory tests. This is particularly important with cholera because in areas where the disease is endemic, cholera may be diagnosed with acceptable accuracy on the basis of clinical symptoms alone. Likewise, cholera may be effectively treated without laboratory confirmation of the etiologic agent. However, early in an outbreak, laboratory confirmation of the etiologic agent is required because other secretory diarrheal illnesses, such as those caused by toxigenic *Escherichia coli* or rotavirus, can mimic cholera. Moreover, because most infections (approximately 75%) with *V. cholerae* O1 are asymptomatic, and cholera gravis (life-threatening dehydration) develops in only about 2% of cholera patients, the diagnosis of cholera may be missed if it is based solely on symptoms, particularly in the early stages of an outbreak or where few cases occur.

During a cholera outbreak, laboratory efforts should be directed toward resolving critical public health issues rather than toward processing a large number of clinical specimens that yield little new information. Laboratory priorities change during the course of a cholera epidemic. In the beginning of an outbreak, confirmation of the etiologic agent is required for suspected cases. Once a cholera epidemic has been established, confirming each clinically diagnosed case is less important, and laboratory efforts should shift to investigating the extent of the epidemic, the source of infection, and the development of antimicrobial resistance. After the epidemic wanes, laboratory confirmation of suspect cases is important for defining the end of the epidemic and guiding public health decisions.

B. When a Threat of Epidemic Cholera is Recognized

In a region threatened by epidemic cholera, the public health laboratory plays a central role in detecting its introduction. Early detection of cholera cases facilitates the selection of appropriate control activities. Laboratory-based surveillance is performed using several types of samples:

1. Clinical samples from "cholera-like illness" at sentinel clinics or hospitals in the absence of a cholera outbreak

Regular sampling of specimens from highly suspect cases can be done periodically, depending on laboratory resources and availability of clinical sites. The definition of suspect cases to be examined for *V. cholerae* should be agreed upon in advance. A patient presenting with severe watery diarrhea and dehydration requiring intravenous therapy should be suspected of having cholera and should be a candidate for surveillance culture. Available resources and the frequency of suspect cases should be

taken into account in performing cultures. Arrangements can be made to collect rectal swabs from all patients with cholera-like illness at several clinical sites, or to sample a subgroup of patients, such as all those with acute diarrhea seen on a particular day of the week or a specific number of patients per month. It is important to plan for an appropriate way of transporting specimens to the laboratory. Examining a limited number of carefully selected specimens is more effective than examining a large number of poorly chosen specimens that may have been improperly handled.

2. Confirmation of cases in the early stages of an outbreak of "cholera-like illness"

Cultures and serologic tests may be done on specimens from household contacts of initial case-patients, and heightened surveillance should be considered for a limited time in the area where an initial case has been confirmed. Such efforts can rapidly determine if the first case was an isolated event or the beginning of an outbreak.

3. Surveillance of sewage collection points

The Moore swab (see Chapter V, "Examination of Environmental Samples") is a simple, reliable, and sensitive method to identify infected individuals in the population served by a sewage collection system. Repeated sampling at central points at 1- to 2-week intervals can efficiently identify infections, symptomatic or asymptomatic, in the area. However, during an epidemic, when swabs are routinely positive, continued sampling offers little additional information and can be discontinued.

4. Laboratory confirmation of *V. cholerae* isolates

If a specimen in the pre-epidemic phase yields *V. cholerae* O1, the isolate should be sent to a reference laboratory for confirmation and characterization. Confirmation of the O1 antigen, the serotype, and the production of cholera toxin is critical for confirming the presence of cholera in the area. A limited number of isolates should be biochemically identified, biotyped, and tested for antimicrobial resistance. Further characterization of isolates, such as molecular subtyping, may help determine the origin of the isolate.

C. During a Cholera Outbreak

Once cholera cases have been confirmed in an area and ongoing transmission is documented either through clinical or environmental surveillance, laboratory efforts aimed at detecting *V. cholerae* O1 infections should be significantly reduced. Not all suspect cases need confirmation, and those that are confirmed do not require extensive characterization. Because the epidemic strain is far more common than nontoxigenic O1 strains, there is little value in testing all isolates for toxin production or

for performing tests other than agglutination with O1 antiserum. Instead, the resources of the public health laboratory should be used for the following:

1. Monitoring development of antimicrobial resistance

Periodic examination of a small number of isolates can detect emerging antimicrobial resistance. Resistance may emerge locally; therefore, small periodic surveys throughout the affected area should be considered.

2. Special epidemiologic investigations

During cholera epidemics, focused field investigations help determine sources of infection and routes of transmission and the rate of spread to family contacts.

Case-control investigations are most precise if both patients and controls can be examined for current or recent infection. Current infection can be documented in patients by culturing specimens from them as they come for treatment. Also, in areas newly affected by an epidemic, serum samples from healthy controls can be analyzed for vibriocidal antibody titers, since potential controls with elevated vibriocidal antibodies indicating recent infection may be excluded from analysis.

Surveys in the households of case-patients can determine if intrafamilial transmission is occurring and interventions, such as household education or household chemoprophylaxis, are warranted.

3. Defining the magnitude of the epidemic and improving the interpretation of surveillance data

Serologic surveys conducted periodically during an epidemic can help determine the number of infections in the population and the proportion that are symptomatic. Results of cultures taken from a sequential series of 50 to 100 patients that meet the case definition used during an epidemic can determine the predictive value of the definition. This will confirm the accuracy of the case definition used for surveillance purposes. If, for example, 80% of patients who meet the case definition have culture-confirmed infection, the predictive value of the definition is high, and patients meeting these criteria may be presumed to have cholera in the absence of culture confirmation.

4. Measuring the impact of control measures

Laboratory surveillance data can determine the effectiveness of control measures. If prevention measures are targeted at specific vehicles of transmission, laboratory tests which document the success or failure of current efforts to disinfect those vehicles can be used as indicators of the efficiency of specific control measures, rather than simply culturing vehicles for *V. cholerae* O1. Evidence of adequate chlorination of water supplies and documented absence of fecal coliforms in water, foods, and

beverages are more reassuring than the failure to detect *V. cholerae* O1 in samples of these items.

D. Defining the Duration of the Epidemic

A substantial decrease in the number of clinically defined cholera cases in a population may represent a temporary seasonal decline, transition to an endemic state, or complete disappearance of cholera from the population. Transmission may cease altogether in some regions, and persist at lower levels in others. Because epidemic cholera often decreases sharply in cooler seasons and returns the following summer, declarations that an area is cholera-free may be premature. At this point, targeted laboratory surveillance can help define the situation. As in the pre-epidemic period, periodic sampling of persons with severe "cholera-like illness" and of central sewage collection points can again be used with great sensitivity to determine the presence or absence of the epidemic strain. Not until 12 months have passed without evidence of *V. cholerae* O1 can an area be declared cholera-free with confidence. A report can state that no cases of cholera have been detected since a specific date without declaring that an area is cholera-free.

E. Special Problems

1. Retrospective diagnosis of a suspected outbreak

If a population is reached after a suspected cholera outbreak has subsided, serum samples may be collected for assay of vibriocidal antibody titers. Sera may be collected from a limited number of "typical" patients and healthy controls. Collecting control sera in the same village permits comparison of vibriocidal titers of the "ill" group and the "healthy" group. The choice of serologic assay depends on the timing of specimen collection (see Chapter VIII, "Detection of Patient Antibodies to *V. cholerae* O1 and Cholera Toxin"). Vibriocidal titers begin to rise several days after exposure, usually peaking by 10 to 21 days, begining to decline within 1 month, and returning to baseline levels after about 1 year. Anti-cholera toxin antibodies peak 21 to 28 days after exposure and remain elevated for more than a year after infection.

2. Environmental sampling

Methods for sampling foods, water, and other environmental specimens for *V. cholerae* O1 are labor-intensive and relatively insensitive and can rapidly deplete laboratory resources without yielding clearly interpretable results. Culture-negative environmental samples may mean that the specimens were collected too late or were mishandled. If several different types of samples yield *V. cholerae* O1, it may not be clear whether the food or water caused the illness or were contaminated by the infected persons.

In general, broad environmental surveys are not recommended. However, if a specific vehicle of transmission has been identified epidemiologically, targeted sampling of that suspected vehicle can yield useful information. Similarly, once control measures have been taken to reduce the contamination of the vehicle, microbiologic assessment can determine the success of the intervention.

F. Summary

In summary, the public health laboratory can provide critical information in defining the beginning of a cholera epidemic, monitoring resistance and other changes in the epidemic strain, and defining the course of the epidemic. Collaborating with epidemiologists, the public health microbiologist can support efforts to determine the sources of infection and measure the effectiveness of control measures. Many critical questions can be answered by careful use of laboratory resources. Clarifying the precise questions to be answered, and giving careful attention to sample selection, specimen transportation, and the efficient use of diagnostic tests can prevent depletion of laboratory resources by tests of questionable epidemiologic value.

Reference

Global Task Force on Cholera Control. Guidelines for cholera control. Geneva: World Health Organization; 1992. publication no. WHO/CDD/SER/80.4 Rev 4.