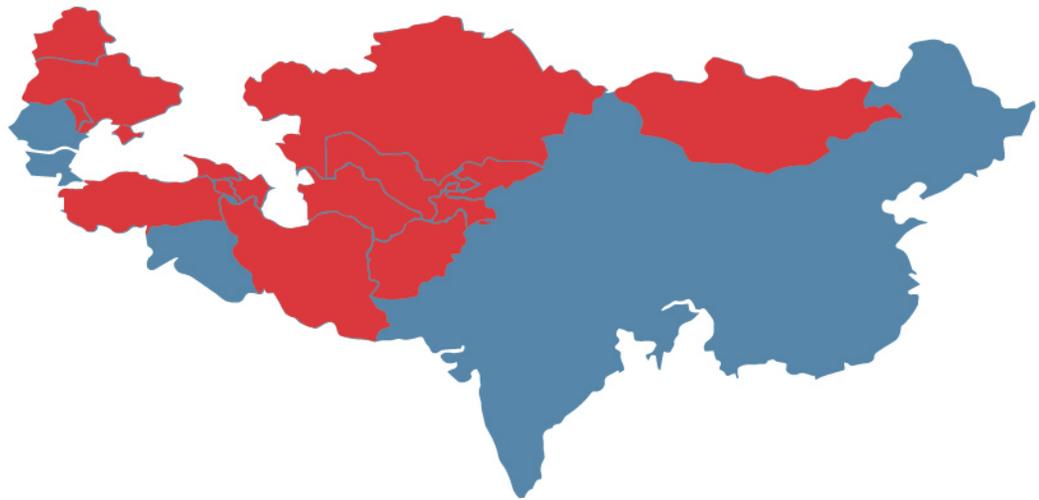


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Suspected Cholera Outbreak in Wardak Province, Afghanistan, 2022: A Teaching Case Study of Laboratory Role in Outbreaks Investigation

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Abstract

This teaching case study highlights the crucial role that laboratories play in the investigation and management of disease outbreaks. Designed specifically for participants in the intermediate tier of the Field Epidemiology Training Program (FETP), the case study aims to deepen the understanding of laboratory contributions to outbreak response efforts. By focusing on a real-life outbreak scenario, it provides practical, hands-on learning experiences that bridge theoretical knowledge with field applications. The case study is grounded in an actual outbreak of Acute Watery Diarrhea (AWD) that affected two villages in the Jalrez District of Wardak province in July 2022.

The primary objective of this teaching case study is to educate FETP residents about the significance of laboratories in outbreak investigations. By exploring the AWD outbreak, participants will learn about various aspects of laboratory involvement, including specimen types, collection procedures, and proper management of collected samples.

Key Learning Points:

1. Laboratory's Role in Outbreak Investigations: Participants will gain insight into how laboratories play a critical role in confirming outbreaks, identifying pathogens, and guiding control measures. They will understand the importance of accurate and timely laboratory results in informing public health decisions.

2. Specimen Types and Collection: The case study delves into the different types of specimens used in outbreak investigations, with a focus on AWD. Participants will be challenged to discuss on which specimens are most appropriate for diagnosing specific diseases and the significance of sample timing in obtaining reliable results.

3. Specimen Collection Procedures: FETP residents will be guided through proper specimen collection techniques, emphasizing the importance of maintaining sample integrity to ensure accurate laboratory analysis. They will learn how to minimize contamination risks and ensure representative sampling.

4. Specimen Management: This section highlights the significance of proper specimen management, including storage, transportation, and documentation.

5. Laboratory-Field Collaboration: The case study underscores the importance of effective communication and collaboration between field epidemiologists and laboratory personnel. Participants will learn how to facilitate seamless information exchange and coordination to ensure efficient outbreak response.

6. Real-life Application: By examining the AWD outbreak in two villages of the Jalrez District, participants will gain practical insights into the challenges and complexities of real-world outbreak investigations. This context allows for a comprehensive understanding of the concepts discussed.

7. Public Health Decision-Making: Through the lens of the AWD outbreak, FETP residents will explore how laboratory results influence public health decisions, from implementing control measures to resource allocation.

Time allotted: 1-2 Hours

Language: English

Groups: 3-5 residents per group

Materials Required: Pen, pencil, flip chart, calculator, marker, highlighter pen, and personal computer (if available)

Introduction

According to definition of Cholera Global Task Force, Acute Watery Diarrhea (AWD) is defined as an illness lasting less than seven days with non-bloody liquid stools which may contain mucous and defecating frequencies of three or more loose stools in less than 24-hours [1]. In some contexts, AWD will be accompanied with severe dehydration which can be defined as a person having AWD and manifesting lethargy, loss of consciousness, absence or weak pulse and respiratory distress OR having at least two signs from sunken eyes, inability to drink water, skin turgor (skin pinch going back in slow manner or in more than two seconds) [2]. Majority of AWD cases are associated with Cholera infection. This infection is caused by Vibrio Cholera bacterium with wide range of serotypes while O1 and O139 serotypes are mainly responsible for cholera outbreaks. Frequently observed indications of Acute Watery Diarrhea (AWD) due to cholera encompass diarrhea, abdominal unease, and vomiting. In more severe cases of cholera, hypovolemia emerges, resulting in fluid depletion, parched oral mucosa, reduced skin elasticity, and disrupted electrolyte equilibrium. One of the main transmission routes of cholera is water sources infected with the bacterium. Cholera constitutes a worldwide issue concerning public health and is indicative of a country's inadequate development [3].

Instances of Cholera Outbreaks have impacted numerous nations in recent years, primarily across Asia and Africa. Countries including India, Sudan, Pakistan, Bangladesh, and Afghanistan have experienced such outbreaks [4]. Since January to August 2023, a total number of 128,880 AWD cases have been reported from 331 districts of Afghanistan with more than half of the cases being occurred among children under the age of five years. Additionally, a total number of [5].

Part-I Story

Wardak province located in central-west region of Afghanistan, has 9 district and in 30 Kms of Kabul. The National Diseases Surveillance and Response (NDSR) department of Wardak Provincial Public Health Directorate (PPHD) received initial reports from Zewalat and Folad Khan villages of Jalrez district regarding unexpected increase in diarrhea cases on July 21st, 2022. The Emergency Preparedness and Response (EPR) committee meeting commenced consequently to prepare for investigation of this outbreak. The team consists of NDSR officer, Communicable Diseases Control (CDC) officer and Surveillance Support Team (SST) staff including medical doctors and laboratory technician. They eventually moved to the field and started their investigations and response. The results of primary assessment of the situation indicated cases with AWD clinical manifestations and suspected cholera outbreak. For management and containment of this outbreak, the team was ought to make their suspicions to belief. Therefore,

the team reached to the conclusion of drawing biological specimen from suspected cases to confirm the etiological agent of this outbreak.

Question-1: What are Public Health Laboratories (PHL) and their core functions?

Question-2: What are the main surveillance PHLs in Afghanistan health infrastructure and in which capacity do they function?

Question-3: What are the main common uses of laboratory data for public health surveillance?

Part-II Methods

The investigation team used Ministry of Public Health's (MoPH) case definition as of below:

"The NDSR Department of MoPH defines AWD as an illness characterized by 3 or more loose or watery (non-bloody) stools within 24-hour with or without dehydration."

The team has line listed a total of 33 cases during July 21st to July 27th using the above case definition. The cases were peaked during July 21st to July 23rd with 20 cases (Figure 1). As the cases have clinical representation of AWD and the team is suspected of cholera, SST team is prepared for specimens' collection. Typically, most specimen collection that occurs during a disease outbreak involves collecting human clinical specimens from outbreak-associated case patients. Environmental specimens are frequently collected in conjunction with human clinical samples; when outbreak associated clinical and environmental specimens yield the same results, the hypothesis that the outbreak source is the same as the environmental specimen source can be supported. During outbreak investigations, both clinical and environmental specimens are collected and analyzed to understand the source, transmission, and extent of the outbreak based on context. The main types of clinical specimens include blood, urine, sputum, feces, serum and may differ based on the context of outbreak. The similar type of specimens could be drawn from the infected animals as well. Moreover, the main types of environmental specimens include foods, water, fomites, surfaces and more.

The cholera bacterium can be found in stool of suspected individuals manifesting clinical symptoms of cholera infection. If the stool specimen could not be processed, a rectal swab is advised to be collected from suspected individuals. Fecal samples (liquid stool or rectal swabs) ought to be gathered during the initial phase of the sickness, as this is when pathogens are

typically most abundant in the stool, specifically within the initial four days of the illness. Considering preventive measures during specimen collection, the assigned team are required to collect the specimens in specified containers and would be placed in transport mediums in case of tests being performed on the specimens.

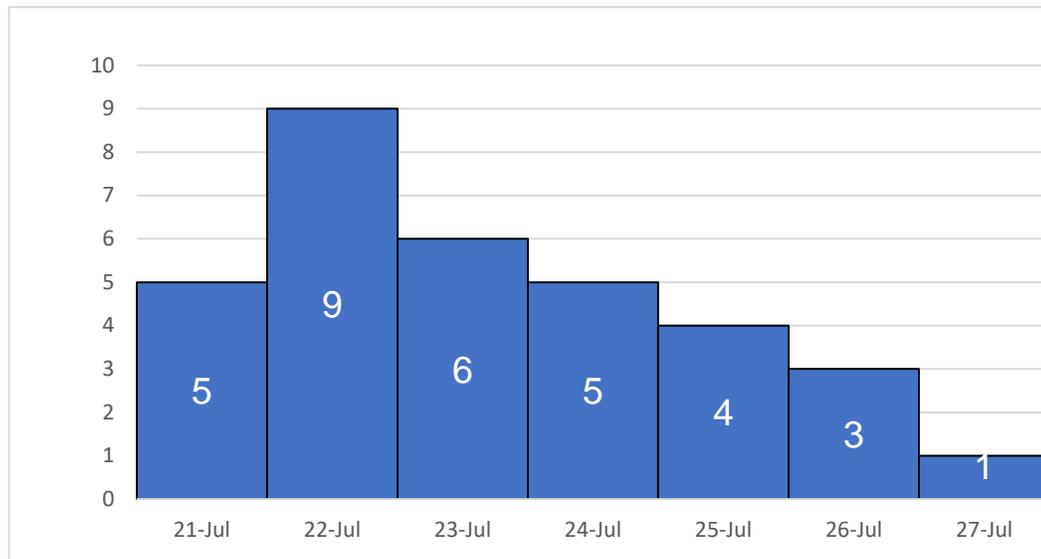


Figure 1 Epidemic curve of AWD outbreak in Zewalat and Foad Khan villages of Jalrez district, Wardak Province

Question-4: Which containers are usually used to collect following clinical specimens?

- 1- Urine specimens:**
- 2- Blood specimens:**
- 3- Stool specimens:**
- 4- Swab specimen:**
- 5- Respiratory specimen:**

Question-5: a) What's PPE kits?
b) Which equipment are included in PPE kit?

The investigation team collected three stool specimens from suspected individuals along with one water source sample. The samples were then tested for presence of cholera bacterium antigen on the field promptly using cholera Rapid Diagnostic Test (RDT) diagnostic kits. All of the collected specimens were positive for presence of cholera antigen. Moreover, the water specimen was tested positive for cholera antigen using RDT. As recommended by Cholera Global Taskforce where available the RDT could be helpful in confirming the outbreak. However, RDT+ specimens should be confirmed by other cholera diagnostic tests as of below:

- Culture or seroagglutination tests for identifying species and serogroup of the cholera
OR
- PCR for species and serotype identification

The provincial hospital of Wardak province (Maidanshahr PH) is not equipped with adequate facilities to process the collected specimen for confirmation of RDT+ samples. The nearest facility to process this sample is Kabul's Central Public Health Laboratories (CPHL). This laboratory is equipped to culture the clinical specimen for determining presence of cholera bacterium and its' serotype. However, prior to emergence of this outbreak, the highways connecting Wardak to Kabul were somehow damaged and floods made it worse. Therefore, despite not being very distanced from Kabul, the specimen delivery from Wardak could take more than two hours. Hence, the collected specimens should be properly packaged and shipped to Kabul CPHL for confirmation of the outbreak in timely manner.

Question-6: What are transport mediums?

Question-7: What are the most common transport mediums for stool, viral respiratory and blood specimens?

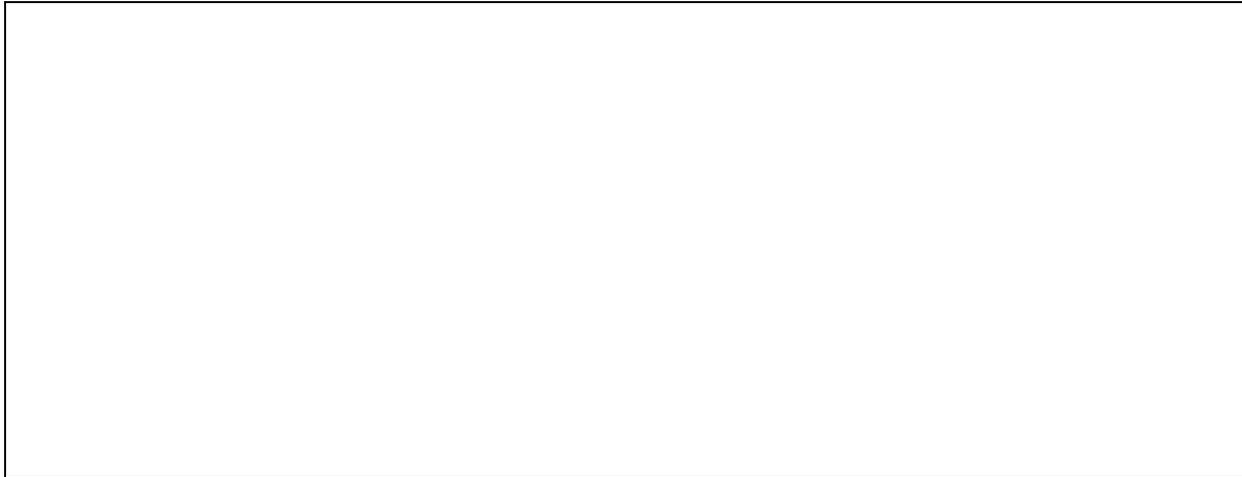


The investigation team prepared the specimen for transport and selected Cary-Blair as the recommended transport medium. However, to safely transport the specimen and accurate delivery, specimens should be labeled, packaged and shipped properly. Therefore, in each single step ultimate precaution is required.

Question-8: What is the importance of labeling on specimens and which type of information is included in labels?



Question-9: Some clinical specimens (including cholera specimens) are transported in **triple packages** designed to ensure the safety of specimen throughout shipping. Describe the structure of triple package and draw a basic illustration with outline of its main parts.



The clinical specimens were labeled properly and packaged using triple package method. Currently these specimens are readily available for transport. Considering the time of the year during transport (July is midst of summer in Afghanistan), the specimens should be transported in safe condition and precise temperature to prevent any sort of specimens degradation. Therefore, specimen carrier is used to transport clinical specimens from field to laboratory. This carrier is box-shaped and maintains the temperature between 4-8 centigrade. This specimen carrier is valid to transport the clinical specimen for 24-48 hours. For the specimens with longer time window, freezing in minus 70 centigrade is recommended. The specimen was carefully packaged and placed in a specimen carrier, ready for transportation to Kabul's CPHL. Prior to transport, meticulous preparations were undertaken to ensure the specimen's safety during transit. The route and mode of transportation were thoughtfully selected. Once safely arrived at the CPHL in Kabul, the necessary diagnostic procedures were promptly administered.

Part-III

Results

To confirm the cholera outbreak in RDT+ samples, the recommended method of specimen culture was employed. The three specimens were subjected to culture using Thiosulfate Citrate Bile Sucrose (TCBS) selective agar, a widely adopted medium for *Vibrio Cholera* bacterium isolation. This particular agar type is not only easily obtainable but also simple to prepare, eliminating the need for autoclaving. Its distinguishing feature lies in its exceptional differential and selective properties. Upon preparation, TCBS agar showcases a distinct green color.

Following an overnight incubation period with the inoculated specimen (lasting from 18 to 24 hours), the growth of *V. cholerae* becomes evident, presenting as substantial yellow colonies

with slightly flattened forms, measuring 2 to 4 mm in diameter. These colonies exhibit opaque centers surrounded by translucent peripheries. The yellow coloration stems from sucrose fermentation within the medium. Remarkably, the culture of all specimens produced positive results, confirming the presence of the cholera bacterium.



Figure 2: Cholera bacterium growth on TCBS selective agar

For serotyping, anti-sera containing antibodies tailored to the O-antigen of the cholera bacterium were employed. The anti-sera interacted with the O-antigen, confirming the cholera bacterium's serotype as Ogawa. Therefore, all three stool specimens and one water sample were confirmed for cholera bacterium and the serotype was determined as Ogawa. Consequently, further collaboration with Wardak PPHD was established to communicate the cholera outbreak confirmation in Zewalat and Folad Khan villages of the Jalrez district. Upon receiving confirmation, Wardak PPHD proactively addressed this outbreak with focused efforts on prevention in neighboring districts of Wardak province. While control measures effectively contained the outbreak in the mentioned villages, public awareness about hygiene practices remained a priority. Given the positive cholera test in the water source specimen, thorough chlorination of wells was conducted. In addition to health education, distribution of soaps and essential hygiene kits was carried out among the residents of these villages. The total number of cases reached a toll of 30 by the end of the outbreak and no deaths were recorded during this period. The outbreak was controlled specifically in both villages by July 29th.

Part-IV

Conclusion

Question-10: Write conclusion and recommendations based on findings of this outbreak investigation.

Acknowledgment

Afghanistan Field Epidemiology Training Program (AFETP) extend their gratitude to National Diseases Surveillance and Response (NDSR) department of Wardak Provincial Public Health Directorate, Central Public Health Laboratories of Afghanistan under the roof of Monitoring, Evaluation and Health Information System (M&EHIS) General Directorate of Ministry of Public Health for providing data and information of this teaching case study.

Conflict of Interest Statement

The authors declare no conflict of interest related to this manuscript.

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