

A NOVEL STRAIN OF *SHIGELLA* SPECIES OUTBREAK IN A RESIDENTIAL SCHOOL IN PEMAGATSHEL, BHUTAN, 2012

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Accepted: 1 December 2016

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ABSTRACT

Background: A cluster of suspected shigellosis was reported from health center in Pemagatshel district to Royal Center for Disease Control on 14th May 2012. The investigation was done to determine the cause and risk factor for the outbreak so that appropriate control and prevention measures can be implemented.

Methods: A descriptive study was used for the outbreak investigation. The food items and drinks served to boarding students were collected from the mess in-charge in order to assess their risk for the outbreak. The kitchen and its premises were inspected to study the likely contamination by rodents and other animals. The water and stool specimens were tested in the laboratory to identify all possible enteric pathogens.

Results: 82 boarding students were affected with an overall attack rate of 28% (82/294). Diarrhea was the predominant symptom followed by abdominal pain and headache. The onset date of the cases varied between 11th and 18th May, 2012. *Shigella* species was isolated from stool specimens that showed resistant to amoxicillin, nalidixic acid, chloramphenicol and sulfamethoxazole. Water specimen collected from source, distribution reservoir and tap water at school were found grossly contaminated.

Conclusion: The outbreak was caused by novel strain of *Shigella* species which was not detected earlier in Bhutan. The promotion and provision of boiled water will greatly reduce the incidence of shigellosis especially in boarding facility.

Key words: Shigellosis; MDR-Shigella; Boarding school; Bhutan

INTRODUCTION

Shigellosis or bacillary dysentery is an acute gastroenteritis which occurs in areas with crowding and poor sanitary

conditions.¹ *Shigella* species are the major etiological agents of bacillary dysentery.² Globally, it is estimated that 164.7 million people are infected annually by *Shigella*

often through contaminated food or water.^{1,3} *Shigella* serogroups are considered to be highly infectious due to their low infectious dose (10 – 100 organisms).^{4,5} Dysentery bacillus encompasses four serogroups namely *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*. Each of these is composed of different serotypes, which are identified based on the structure of lipopolysaccharide O-antigen repeats: *Shigella dysenteriae* has 15 serotypes, *Shigella flexneri*, 14 serotypes, *Shigella boydii*, 20 serotypes and *Shigella sonnei*, a single serotype.^{6,7}

Shigellosis is a national notifiable disease that every health centers in the country has to notify to Public Health Laboratory, now renamed as Royal Center for Disease Control (RCDC) for verification and activating an outbreak response.⁸ Annually, suspected shigellosis is reported sporadically from many of the health centers in Bhutan.^{9,10} On 14th May, 2012, a cluster of suspected shigellosis among boarding students of Nganglam Higher Secondary School (NHSS) in Pemagatshel district was reported to RCDC by medical officer of Basic Health Unit 1 (BHU – 1). A team from RCDC was sent to school on 16th May, 2012 to investigate an outbreak. The investigation was conducted to determine the etiological agent of suspected shigellosis and their concomitant study of antimicrobial susceptibility pattern. The investigation was also aimed to trace the source of outbreak so that appropriate control measures are implemented to prevent from further spread to general population in the community.

METHODS

Epidemiological investigation

A descriptive study was used for the investigation of outbreak. A suspected case was defined as any boarding students

studying in Nganglam Higher Secondary School (NHSS) in Pemagatshel district, Bhutan with clinical manifestation of diarrhea with or without abdominal pain, nausea, vomiting or fever from 10th to 18th May, 2012. All school students and teachers were assembled in the dining hall with permission from the Principal to seek active case finding.

The food items and drinks served to boarding students in the past few days were collected from the mess in-charge. The personal hygiene and stool specimens of the cooks were also examined. Face to face interview was conducted among all cases to study the exposure of food items in the past few days.

Environmental investigation

The hygiene of the kitchen and its premises was inspected by the team. The store where vegetables and other culinary items were stacked was also inspected to study likely contamination by rodents and other possible sources. The team visited school water source to inspect surrounding sanitation and collect water samples. Water samples were collected from reservoir, distribution tank, and taps from kitchen, boys and girls hostel for testing indicator bacteria using fecal coliform medium (mFC broth).¹¹

Microbiological investigation

A total 12 stool specimens from both hospitalized and outpatient unit were collected and subjected to standard microbiological test. Both macroscopic and microscopic examination was done for all the collected specimens. For microscopic examination, a wet mount was prepared using 0.85% Normal Saline and observed using a light microscope for cells, ova and parasites. Furthermore, the same specimens were processed for culture and identification of bacterial pathogens. Briefly, suspensions of stool specimens were made in 0.85% Normal Saline. The suspension was enriched in Buffered

Peptone Water (BPW), Alkaline Peptone Water (APW) and Preston, and plated on Mac-Conkey Agar, Hecton Enteric Agar and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA). All media were incubated aerobically except for mCCDA which was incubated at microaerophilic atmosphere at 37°C. The colonies from each media were identified biochemically using Kligler Iron Agar (KIA), indole, bile esculin, lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase and also by using bioMerieux Analytical Profile Index (API) 20-E. Identified organism was then subjected to antimicrobial susceptibility testing using Clinical Laboratory Standard Institute (CLSI) guideline.¹²

Statistical analysis

The demography of the cases and antimicrobial susceptibility pattern of bacterial pathogen were presented in terms of numbers and percentages. The distributions of cases were presented graphically by using their date of onset of illness. Ethical clearance from Research Ethics Board of Health (REBH), Ministry of Health, Bhutan was not required for the investigation conducted in response to disease outbreaks.

RESULTS

Epidemiological investigation

The school had a total of 482 students out of which 294 are boarding students (110 girls and 184 boys). About 82 boarding students were affected in the outbreak with an overall attack rate of 28% (82/294). Sex specific attack rate was observed high among girls (40%) than boys (20%). The median age of affected students was 17 years which ranged from 15 - 21 years. Diarrhea was the predominant symptom observed in all cases. The other symptoms included

abdominal pain, nausea, vomiting, fever and headache in this order (Table 1).

Table 1. Symptoms of cases in an outbreak of suspected shigellosis in NHSS, 2012 (n = 82)

Symptoms	Number	Percentage (%)
Diarrhea	82	100.0
Abdominal pain	65	79.2
Headache	45	54.8
Fever	44	53.6
Dysentery	41	50
Nausea	32	39.0
Vomiting	20	24.3

The index case was detected on 13th May 2012 in health center. However, on active case finding, the cases actually emerged on 11th May but they didn't seek medical care and were not detected in the health center. The onset date was between 11th May and 18th May 2012 with majority of the cases reporting their onset of symptoms on 13th May. No case was detected on 15th May. With one case on each 16th and 17th May, the case rose to five on 18th May and thereafter, no cases were reported (Figure 1).

None of the students were reported to have consumed food from nearby commercial shops or restaurants. All boarding students had consumed same food items provided in the mess. As per the information provided by the students, foods provided in the mess were cooked adequately and no hint was ever reported on the possibility of food contamination. Even the personal hygiene of all four cooks was found satisfactory on screening their health. However they pointed out that the consumption of un-boiled water could be the risk for the outbreak as they are not provided with boiled water in the boarding facility (student hostel).

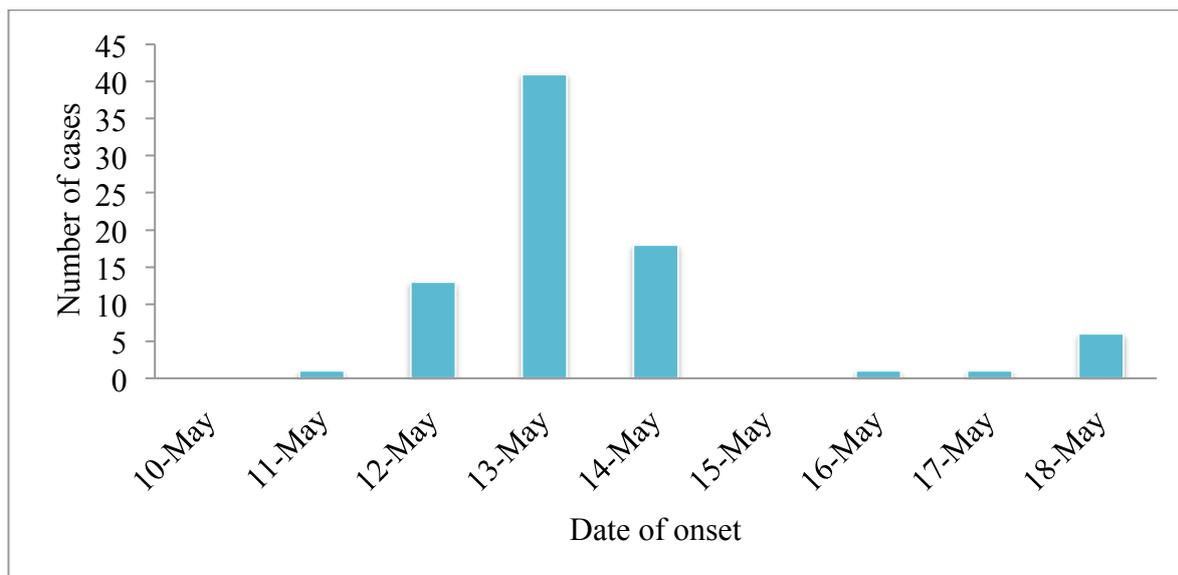


Figure 1. Epidemic curve of shigellosis outbreak in NHSS, 2012, by date of onset

Environmental investigation

The kitchen and its premises were found hygienic. Vegetables and other culinary items were also properly stacked in kitchen-store with no trace of rodents. Water source was located around 20 - 30 minutes' walk from the school. Water source for the school was a running stream

in the forest connected to reservoir. During inspection, the surrounding of water source was found contaminated with cattle feces with few cattle grazing near it. Water specimen collected from source, distribution reservoir and tap water at school were found grossly contaminated. (Table 2)

Table 2. Water quality test results at different water sources in NHSS, 2012

Sampling points	Test results	Interpretation
School reservoir tank	> 50 CFU/100 ml	Grossly polluted
Distribution tank	> 50 CFU/100 ml	Grossly polluted
Boys hostel tap water	20 CFU/100 ml	High health risk
Girls hostel tap water	10 CFU/100 ml	Intermediate health risk
Kitchen tap water	26 CFU/100 ml	High health risk

Note: CFU = Colony forming unit; ml = milliliter

Microbiological Result

Six stool specimens were collected from each of the hospitalized and non-hospitalized cases. Mucus and blood were visible in nearly half of the collected stool specimens. Red blood cells and white blood cells were found in all the specimens.

From culture and biochemical test, *Shigella* was also isolated from five of 12

stool specimens. The identification was further confirmed by Analytical Profile Index (API) 20 E as "highly pathogenic shigella species". However on serotyping, none of the isolates were agglutinated with all available antisera (DENKA SEIKEN, JAPAN) and additional antisera of *S. dysenteriae* serotype 13, 14 and 15 (Reagensia AB, Solna, Sweden). The antibiotic susceptibility test showed that all

isolates were susceptible to cefazolin, ciprofloxacin, cephalixin, ceftriaxone and gentamycin, and resistant to amoxicillin and nalidixic acid. Furthermore, except for one isolates, the four other isolates were resistant to chloramphenicol and sulfamethoxazole.

DISCUSSIONS

This is the first documented outbreak of shigellosis in the country caused by novel strain of *Shigella* species which could not be serotyped with the available antisera. The past outbreaks in Bhutan are mostly caused by either *Shigella sonnei* or *Shigella flexneri* which were confirmed by serotyping with the existing antisera⁸⁻¹⁰. The clinical manifestation caused by serogroups of *Shigella* is similar with mucus and bloody diarrhea¹³. However, the dysentery caused by *S. dysenteriae* is severe as compared to other serogroups¹⁴. The current outbreak is most likely caused by *S. dysenteriae* based on non-fermentation of mannitol although isolates tested negative with all antisera. Moreover, the current outbreak has caused hospitalization of the cases due to loss of body fluids and severe dehydration. With timely intervention in the health center, no case fatality was reported in the outbreak.

Except for one pathogen, the remaining four pathogens were resistant to amoxicillin, chloramphenicol, sulfamethoxazole and tetracycline. Such Multi-Drug Resistant *Shigella* (resistant to three or more antibiotics) was also reported in Nepal, Africa, India and Zimbabwe¹⁵⁻¹⁹. An emergence of MDR-*Shigella* might be associated with the irrational or overuse of antibiotics in the healthcare facilities.

All cases were residing in the boarding facility with preponderance of girls. The exclusion of cases among dayscholar students suggests that they have more leverage than boarding students on their style of dietary habits. Moreover, they

also reported that they drink boiled or filtered water all the time, whereas, boarding students were not supplied with such facilities at the hostel.

A single isolation of non-serotyping *Shigella* species from the stool specimens suggest that all cases were exposed to same source. Drinking water could possibly be the source of this outbreak because all water specimens tested at different sampling units were contaminated. This finding was also supported by the number of cases detected on different dates which indicates that they might be exposed to contaminated water at different time periods. The epidemic curve shows a difference of seven days between the first and the last case. If it was a common point source outbreak, all cases would have their onset within 1-3 days after infection because the incubation period of *Shigella* is 1-3 days²⁰. The isolation of *Shigella* from water specimens would have confirmed the source of outbreak but RCDC do not have those sophisticated testing facility for water and other environmental specimens for isolating bacterial pathogens.

Interventions taken

Water tanks that supplied water to school was cleaned thoroughly and provision of boiled water in boarding facility was suggested. Health education was also given to all students and teachers on the transmission and prevention of shigellosis.

CONCLUSIONS

The outbreak is caused by novel strain of *Shigella* species which was not detected in any health centers of Bhutan. Surveillance on bacillary dysentery has to be continued to monitor the distribution of serogroups and their antimicrobial susceptibility pattern to guide the treatment of patients. School administration should

provide enough boiled water for drinking purpose in school including hostels.

ACKNOWLEDGMENT

The team thanks all students, teachers and cooks of Nganglam Higher Secondary School for providing candid responses during the investigation. The team also thanks Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand for helping us confirm the bacterial isolates.

Conflict of interest

The authors declare no conflict of interest.

Funding

Not applicable

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Cite this article as: Tsheten, Tshering D, Gyem K, Dorji S, Wangchuk S, Irfani TH, Letho. A Novel Strain of Shigella Species Outbreak in A Residential School in Pemagatshel, Bhutan, 2012. *Public Health of Indonesia* 2016; 2(4):165-171