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An Outbreak of Shigellosis in a Remote Village of Mongar District, Bhutan from March to April 2011

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Abstract

On 26 Mar 2011, staff at Mongar Hospital in Bhutan was notified of an outbreak of bloody diarrhea in a remote village. An investigation was conducted to determine the magnitude of outbreak and the source of infection. A case was defined as an individual in the village who developed diarrhea between 18 Mar 2011 and 3 Apr 2011. Active case finding and an environmental survey were conducted in the village. Laboratory investigations of fecal and environmental samples were carried out. Of 94 people residing in the village, 38 (40.4%) met the case definition. One case died, giving the case fatality ratio of 2.6%. The attack rate among females (51.1%) was significantly higher than males (30.6%). The secondary attack rate within households was 25.0%. *Shigella flexneri* was isolated from two of 10 stool samples. Poor sanitation and inadequate hygiene were observed in the village. Water samples were found to have fecal contamination. By multiple logistic regression analysis, significant risk factors for contracting the disease included being female, having no latrine as well as visiting and eating food in a sick neighbor's house. *Shigella flexneri* was the probable cause of the bloody diarrhea. Contaminated water might be the primary source of this enteric pathogen. Decontamination of water and improvement in hygiene might curtail future spread of the infection.

Keywords: shigellosis outbreak, *Shigella flexneri*, contaminated water, Bhutan

Introduction

Shigellosis, commonly manifested by bloody diarrhea, is caused by *Shigella sonnei*, *Shigella flexneri*, *Shigella boydii* or *Shigella dysenteriae*. Among the four species, *Shigella flexneri* is the main cause of shigellosis in most developing countries.[1-2] Outbreaks of shigellosis continue to occur in many parts of the world with inadequate water supply, food safety, sanitation and hygiene.[1] The bacteria is shed in the feces of people infected with shigellosis and spread to others when they ingest food or water contaminated with the bacteria. Person-to-person transmission may also occur. Flies also transmit the organism from feces to uncovered food items and, when eaten, may cause infection.[1-2]

Diarrheal diseases remain among the top ten most prevalent diseases in Bhutan. The overall incidence of diarrheal diseases in Bhutan during 2010 was 126.6 per 1,000 people. There have been no representative data on shigellosis; however, it has been a notifiable disease in Bhutan since May 2010.[3] In Mongar District, diarrhea was among the top five causes of morbidity.[4] The incidence in Mongar during 2010 was 103.4 per 1,000 people and was higher among children under five years old (276.2 per 1,000). Dysentery contributed about 30% of diarrheal morbidity in Mongar. In 2010, the laboratory in Mongar Hospital cultured *Salmonella* and *Shigella* species from 250 stool samples from patients with dysentery. Of which, *S. flexneri* was the most common organism isolated (3.2%).

On 26 Mar 2011, an unusual rise of bloody diarrhea with death of a child was reported to Mongar Hospital by people of the Dak Village. Dak is a small village located in a remote part of Mongar District, eastern Bhutan (Figure 1). It had approximately 100 inhabitants who were mainly farmers living in 27 households. It was linked by a mule track that passed through a jungle and mountains. The nearest health care centre is in Silambe, which is situated about two-hours walking distance from the Dak Village. The

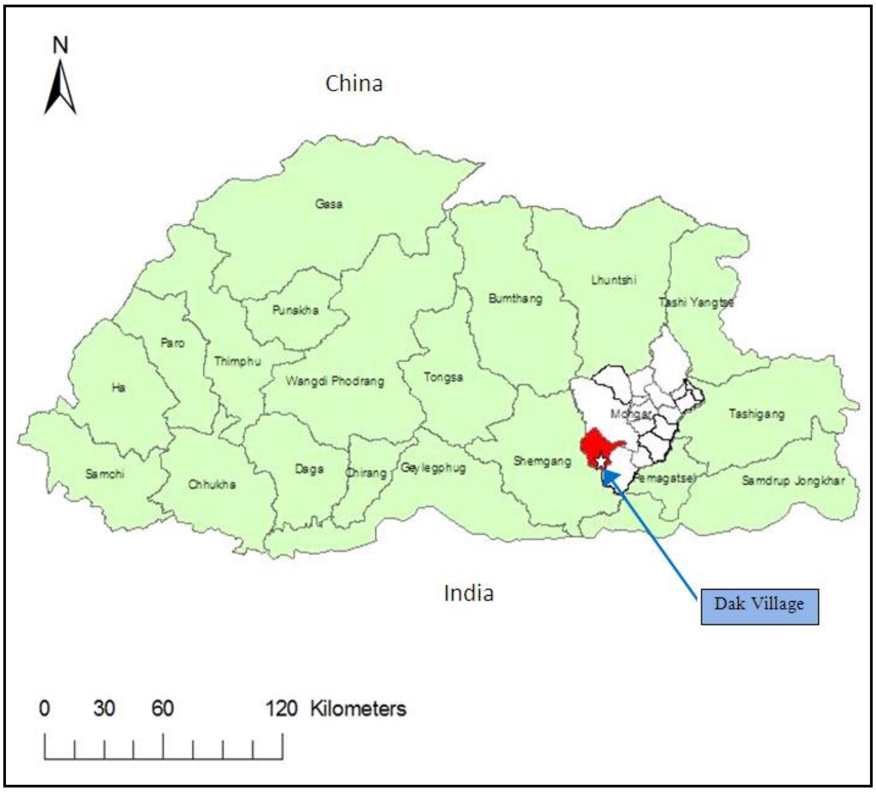

Figure 1. Map of Bhutan showing location of Dak Village in Mongar District

investigation team from Mongar Hospital and local health workers from Silambe Health Centre investigated the outbreak from 27 Mar to 1 Apr 2011. The investigation was conducted to confirm the diagnosis, describe epidemiological characteristics of the outbreak, identify risk factors, locate source of infection and provide recommendations for prevention and control of the outbreak.

Methods

Descriptive Study

The investigation team reviewed the monthly reports for diarrhea and dysentery from the Silambe Health Centre and Mongar District Health Office from January 2006 to April 2011. In Bhutan, diarrhea was defined as passing three or more loose stool over 24 hours with or without dehydration while blood dysentery was defined as diarrhea with visible blood in the stool. Active case finding was conducted by visiting all the houses and interviewing the people lived in Dak Village during the study period. A suspected case was defined as an individual living in Dak Village who developed diarrhea between 18 Mar and 3 Apr 2011. A confirmed case was a suspected case with bacteriological confirmation of a causative organism by culture or serological test.

To describe the transmission among household contacts, we evaluated the secondary attack rate using the following definitions[5]. A household contact was defined as a person living in the same house as the index patient in the household. An index case for the household was a suspected or confirmed case that had the earliest onset in a household in Dak Village between 18 Mar and 3 Apr 2011. A secondary case was a suspected or confirmed case that had onset of illness within one to seven days after the symptom onset of an index case in the same household.

Laboratory Study

Stool samples were collected for microscopic examination and culture. Stool samples for culture and drug sensitivity testing were inoculated into Xylose Lysine Desoxycholate (XLD) medium and processed immediately. The medium was packed in a cold box (vaccine carrier) which maintained temperature between 4-8°C and transported to the laboratory in Mongar Hospital. *Shigella* strains were sub-cultured on XLD and confirmed by serological testing. Suspected colonies were selected after incubation at 35°C for 24 hours. Antibiotic susceptibility was determined by the disk diffusion method.[6] Total eight antibiotics were tested, including amoxicillin, tetracycline, amikacin, gentamicin, trimethoprim/sulfamethoxazole, cefotaxime, nalidixic acid and ciprofloxacin.

Environmental Study

During household visit in the village for active case finding, we surveyed for environmental hygiene including availability of latrine and waste pit, and presence of flies in domestic and peri-domestic area of houses. Information on hand washing practice, habits related to drinking water, visiting to sick neighbors, attending the gathering event and travel to other villages was collected. We inspected the water system of the village, including drinking water sources, tanks, pipes, taps and water storage in the houses. We collected water samples from source, tank, taps and water containers, and tested for fecal coliform bacteria by the Millipore membrane filtration method in the field.

Analytical Epidemiology

The cohort study was conducted from 27 Mar to 1 Apr 2011 to identify possible risk factors for infection among all the residents of the village. All villagers were interviewed face-to-face using the questionnaire. Cases were suspected and confirmed cases identified from the descriptive study. Non-cases were those who did not report diarrhea during the study period.

Statistical Analysis

Percentage, attack rate, median and range were used for descriptive statistics of the study persons. The chi-square test was used for comparison of proportions. Multiple logistic regression analysis (unconditional method) was used to assess the effect of risk factors, after adjusting for other co-variables. Adjusted Odds Ratios (OR) and the 95% confidence intervals were calculated to indicate the strength of association. All statistical analyses were performed using Epi Info version 3.5.3.

Results

Descriptive Study

From 18 Mar to 1 Apr 2011, total 38 cases met the case definition. This yielded an attack rate of 40.4%. The first case was a 65-year-old female who had onset of illness on 18 Mar 2011. She reported that she did not travel out of the village prior to her illness. Subsequent cases had onset between 22 Mar and 1 Apr 2011 (Figure 2). A five-year-old child with onset on 24 Mar 2011 died after a day of diarrhea and vomiting. Of these patients, 14 (36.8%) were secondary cases within the households. The outbreak affected 22 of the 27 households (81.5%) in the village. The most common symptoms were diarrhea (100.0%), abdominal pain (90.0%), bloody diarrhea (71.1%), vomiting (65.8%), fever (60.5%) and dehydration (31.6%).

Among 38 case-patients, 23 (60.5%) were females and 15 (39.5%) were males. The attack rate among females was 51.1%, which was significantly higher than that of males with 30.6% (p-value 0.04). The outbreak affected all age groups in the village. However, the highest attack rate was among females of 65 years or more (Table 1). The majority of the patients were farmers (68.0%), followed by students (16.0%) and pre-school children (16.0%).

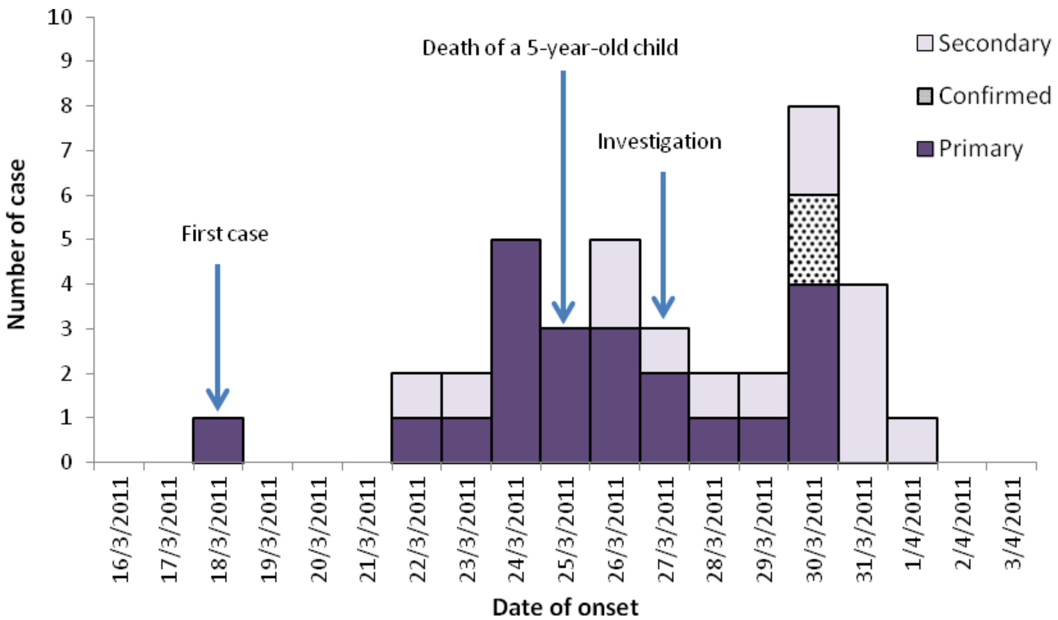

Figure 2. Epidemic curve of diarrhea cases by date of onset and primary or secondary transmission, Dak Village, Mongar, Bhutan, 17 Mar to 3 Apr 2011

Table 1. Attack rate of diarrhea cases by age group and gender in Dak Village, Mongar, Bhutan, 18 Mar to 1 Apr 2011

Age group (year)	Total population		Number of case		Attack rate (%)	
	Male	Female	Male	Female	Male	Female
0-14	15	10	7	5	46.7	50.0
15-44	14	16	3	6	21.4	37.5
45-64	12	12	1	5	8.3	41.7
≥ 65	8	7	4	7	50.0	100.0
Total	**49**	**45**	**15**	**23**	**30.6**	**51.1**

To determine the secondary attack rate within households, we identified 56 contacts in 22 households with a suspected case; 14 subsequently developed diarrhea and thus, had secondary attack rate of 25.0% (Table 2).

Table 2. Secondary attack rate of diarrhea cases by age group of primary cases in Dak Village, Mongar, Bhutan, 18 Mar to 1 Apr 2011

Primary case		Number of population at risk	Number of secondary case	Secondary attack rate (%)
Age group (year)	Number			
0-14	8	23	5*	21.7
15-44	2	8	1	12.5
45-64	7	11	2	18.2
≥ 65	7	14	6	42.9
Total	**24**	**56**	**14**	**25.0**

P-value = 0.41

**All cases were mothers of children with diarrhea.*

Laboratory Findings

Altogether 10 stool samples (five from patients who were already on antibiotic therapy and five from patients who did not receive antibiotics) were transported to the laboratory in Mongar Hospital for culture and drug sensitivity testing. The laboratory tests were performed after five days of stool sample collection. Two of five samples taken from the patients who did not receive antibiotics were found positive for *Shigella flexneri* serotype 2b. Both isolates had identical antibiotic susceptibility pattern which was sensitive to amoxicillin, trimethoprim/ sulfamethoxazole, tetracycline, amikacin, gentamicin, cefotaxime and ciprofloxacin, but resistant to nalidixic acid.

Environmental Findings

The entire village was adequately served by a rural water supply scheme obtained from a running stream. The stream was located in the middle of the jungle. There were both domestic and wild animals found grazing near the source. There was heavy rain from 15 Mar 2011 and lasted until 28 Mar 2011. Water from this source was not treated by chlorine. The water for domestic use was stored in plastic jerry-cans or buckets, and many did not have a lid. The majority (72.3%) of villagers drank water which was not boiled, chlorinated or filtered. Fifteen water samples collected from source, tank, taps and containers showed 30, 30, 32 and 50CFU (Colony Forming Unit) per 100 ml of fecal contamination respectively. The fecal contamination in household water storage containers (50CFU/100ml) was higher than other samples (Table 3).

Table 3. Water samples tested for fecal coliform bacteria in Dak Village, Mongar, Bhutan, 18 Mar to 3 Apr 2011

Type of water sample	Total number tested	Number of fecal contamination	Fecal coliform bacteria per 100ml
Source (running stream)	1	1	30
Tank	1	1	30
Tap	10	10	30
Water container	15	15	40

The assessment of individual households revealed that only 44.4% (12/27) of the households had a latrine for defecation. All were deep pit latrines and there was little possibility of contamination the water supply by latrine waste. No separate hand washing facility was seen near the latrines, yet almost all houses had a water tap nearby. Villagers without a latrine practiced open defecation in pasture land or in the jungle. Of the villagers, 18.3% did not wash hands before eating food and 56.4% did not wash hands after defecation. Similarly, a total of 60 individuals (63.8%) had visited a sick neighbor and of which, 30 (50.0%) got the infection. The infection rate among people who drank unboiled water was 48.4% and that of those who did not have a latrine was 53.7% (Table 4).

Table 4. Potential risk factor for diarrhea in Dak Village, Mongar, Bhutan, 18 Mar to 3 Apr 2011

Risk factor	Exposed		Non-exposed		Risk ratio	95% CI
	Total number	% infected	Total number	% infected		
No latrine at residence	54	53.7	40	22.5	2.4	1.3 - 4.5
Visiting a neighbor with active diarrhea case	60	50.0	34	23.5	2.1	1.1 - 4.1
Drinking unboiled water	66	48.4	28	21.4	2.2	1.1 - 5.1
No washing hands before eating food	17	47.1	77	39.0	1.2	0.7 - 2.1
No washing hands after defecation	53	50.9	41	23.5	1.9	1.1 - 3.4
Attended gathering/funeral	33	45.5	61	37.7	1.2	0.7 - 1.9

Analytic Results

All 94 inhabitants living in Dak Village during the study period were interviewed about hygienic practices. The exposed groups were those who did not have latrine for defecation, drank unboiled water, did not wash their hands before eating and visited the ill. They were compared with the control group who had a latrine, drank boiled water, washed their hands before eating and did not visit people with diarrhea. From the univariate analysis, we found that the risk of contracting the disease among people who did not have latrine was 2.4 times higher than those having latrine for defecation (Table 4). Other risk factors were drinking unboiled water, and visiting and eating food in a sick neighbor's house with diarrhea.

Table 5. Association between potential risk factors and diarrhea by multiple logistic regression analysis, Dak Village, Mongar, Bhutan, 18 Mar to 3 Apr 2011

Risk factor	Adjusted OR*	95% CI	P-value
Age	0.99	1.0 - 1.0	0.40
Gender	3.18	1.1 - 9.0	0.03
Drinking unboiled water	2.41	0.8 - 7.8	0.10
No latrine at residence	3.47	1.2 - 10.1	0.02
No washing hands before eating food	0.37	0.1 - 1.5	0.16
Visiting a neighbor with active diarrhea case	3.90	1.2 - 12.4	0.02

**Adjusted for all variables in the table.*

Multiple logistic regression analysis was used to identify the possible risk factors for infection in this outbreak, after adjusting for other co-variables (Table 5). The significant risk factors included being female, having no latrine as well as visiting and eating food in a sick neighbor's house.

Control Measures

Control measures included active case finding in Dak Village, prompt antibiotic treatment with amoxicillin or trimethoprim/sulfamethoxazole, improvement of hygiene and sanitation in the village including surroundings, latrines and compulsory hand washing before eating and feeding children. The water supply was chlorinated and people were advised to boil all drinking water. The occurrence of new cases had subsequently stopped after five days of implementing the control measures.

Discussion

The diarrhea outbreak occurred during March 2011 in a remote village of Mongar District, Bhutan was probably caused by *Shigella flexneri*. Most of the cases (71.1%) presented with bloody diarrhea while shigellosis is the most common cause of bloody diarrhea in the world.[1] Laboratory analysis of stool cultures confirmed *S. flexneri* in two of 10 patients. Negative stool cultures in five patients were probably due to the antibiotics taken before stool was collected for laboratory analysis. A study in India showed that 83.4% of stool collected from patients after antibiotics therapy had no growth on all cultured media.[7] However, *S. flexneri* was the most common organism isolated from dysentery patients in the laboratory of Mongar Hospital.[4] This organism was reported to be a predominant species in Asian countries.[8-12]

In this outbreak, children and elderly women were predominately affected. Similar age-specific attack rates were reported by a multicentre study of *Shigella* diarrhea in six Asian countries in 2006,[13] a study in a rural village of China in 2005[14] and in other countries[15]. The higher attack rate among young and elderly females might be because females, particularly the elderly, were primarily involved in taking care of the ill family member and might have higher exposure to *Shigella*-containing feces. Another reason for higher susceptibility to shigellosis may be due to less effective immune response of young and elderly people, which could be caused by poor nutritional status and low immunity prior to infection. Poor hygienic practices also favor transmission.

Shigella spreads by eating contaminated food, drinking contaminated water or direct contact with an infected person. The heavy rain in Mongar started in the second week of March 2011, lasted until last week of March and preceded the outbreak. It was quite unusual to have continuous rainfall and a diarrheal outbreak at this period. Normally, the rainy season in Bhutan as well as in Mongar starts in June and lasts till the end of August.[16] The trend of diarrheal diseases also increases correspondingly to the rainy season.[16] The presence of coliform bacteria in water and lack in chlorination were further evidences supporting the contamination of water supply. Fecal contamination could have resulted from human or animal feces, or surface organisms washing into the stream and into open water sources by the rainfall, which is common in mountainous areas.[17] In that report, fecal contaminated water sources in mountainous areas were common because of inadequate source protection, lack of water treatment and poor sanitary practices and resulted in frequent waterborne outbreaks.[17] The appearance of the cases after the rainfall had added our suspicion towards water though it was not statistically significant. On the other hand, poor sanitation and hygiene observed in the village were the clear evidence supporting that the outbreak was related to poor hygiene and sanitary practices. Similar shigellosis outbreaks related to water and poor sanitary practices were also reported in India[18], Thailand[19], Taiwan[20], Greece[21], and Spain[22].

The coliform level in the household water containers was substantially higher than other water sources. This indicated that the water contamination had increased as it reached to the consumption point. Generally, people collected water and stored it in jerry-cans or buckets for several days before refilling. Studies in India during 2008[23] and Bolivia during 2010[24] on quality of drinking water at source and point of consumption found that the practice of water collection, storage, handling, choice of storage container and hygiene significantly affected the water quality in the households.

Person-to-person spread is also the main mode of *Shigella* transmission. The high attack rate of 40.4% among the residents, high secondary attack rate of 25.0% and rapid spread covering 81.4% (22/27) of households in a short period of time suggested that nature of the outbreak was highly contagious. Transmission of infection from person-to-person could have been facilitated by inadequate hand hygiene and frequent contact with a sick person. In this outbreak, having no latrine was one of the significant risk factors. The transmission could have been increased by practice of open defecation that was likely to be the risk for food and water contamination by various disease vectors such as flies, rats and pet animals. The flies and lack in hand washing could have played a vital role in transmitting the organism via the fecal-oral route. Evidence of person-to-person transmission in this study could be further explained by a high secondary attack rate which was higher than reports of studies in Crete[25] and Belgium[26]. Furthermore, *Shigella* may survive up to 8-24 hours in water[27] and in soil at room temperature for 9-12 days[28].

Several limitations of the study were faced because of remoteness of the outbreak village. Firstly, the prevalence of diarrheal diseases before and after the outbreak could not be ascertained due to lack of data and difficulty in returning to the village. Secondly, the number of cases was limited and thus, there was limited detailed analysis of risk factors. Thirdly, we assumed that all secondary cases acquired the infection at home. Alternatively, they might have been infected during school attendance or by visiting sick friends and relatives. Fourthly, we could culture few stool samples as it was difficult in transporting samples due to small capacity of transport facility and long distance to hospital. Lastly, as water source was not treated, we did not test for residual chlorine level in drinking water.

In conclusion, this was the first documented outbreak of a diarrheal disease in the Dak Village, Bhutan. The findings from this study suggested that the poor sanitation and hygiene practice were the main factors attributed to this outbreak. This could be the result of poor living condition of villagers because of low risk perception, low literacy and lack in awareness towards hygiene practice to avert the health crisis. This study highlighted the need of having a pit latrine for defecation in every household and improvement of hygiene as effective measures for controlling future outbreaks. Interventions aimed in improving the living condition of people should go hand-in-hand with sustained health education programs in communities to reduce health risks. Safe water handling, storage practices in households and decontamination of water by boiling need to be emphasized at all levels. The supply of a kit for testing chlorine level of water in district hospitals would greatly benefit in monitoring the quality of drinking water.

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