

ORIGINAL ARTICLE

Investigation of animal anthrax outbreaks in the human–animal interface at risky districts of Bangladesh during 2016–2017

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ABSTRACT

Objective: The objective of the study was to explore the outbreak situation in terms of animal, place, and time towards minimizing the risk of animal infection at the source in future and subsequent spillover in human in the endemic rural settings.

Methodology: An outbreak investigation team from the Department of Livestock Services visited in each of the outbreak sites to explore the event towards strengthening the control program in the future. Meat samples of the infected slaughtered animals were collected to confirm the causal agent of the animal outbreak using polychrome methylene blue microscopic examination technique. Participatory epidemiology tool such as semi-structured interview had been used in these investigations to realize the knowledge and practices of local people/cattle keepers on anthrax control and prevention in animal and human as well.

Results: All identified affected human cases had been confirmed as a history of contact with the animal carcasses or handling/processing with infected meat. The level of awareness at the community level was not satisfactory for the prevention and control of anthrax at the source and further spillover in human. The infected slaughtered animals found to be in non-vaccinated status during the outbreak investigation and uncontrolled animal movement is considered to be responsible for new outbreaks in a vaccinated zone where enforcement of veterinary legislation is inadequate.

Conclusion: A comprehensive preparedness and response strategy is to be obligatory for prevention, control and respond on anthrax in Bangladesh. Maximum vaccination coverage in the animal, increase community awareness of animal and human anthrax are also demanded for transmission of anthrax from animal to human.

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Introduction

Anthrax is an acute infectious disease caused by the bacterium *Bacillus anthracis*, which can affect herbivores animals [1,2]. Animals can obtain infection through contact with soil borne spores of *B. anthracis* while humans obtain infection through contact with diseased animals or with the carcasses during slaughtering or processing infected animal or byproducts of infected animals [1]. Sporadic

occurrence and epizootics of anthrax occur among livestock and wild herbivores in the United States, southern and eastern Europe, and Canada and outbreaks at the animal–human interface are reported from countries in Africa, the Middle East, and Asia [3–8]. In southern Asia, anthrax is considered to be highly enzootic in India and Bangladesh and continual outbreaks occur in both animals and humans [3,5,9].

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In Bangladesh, animal anthrax has been presumed to be enzootic for long before; however, it was recognized as a zoonotic disease in this country during the year 2009–2010 [3]. Anthrax outbreaks have persistent to be recorded in both animal and human from different areas of the country [3]. Anthrax infection in livestock population of Bangladesh has been notified regularly in conformity with the standards set by the World Organization for Animal Health (OIE). In rural areas of Bangladesh, people commonly slaughter sick animals infected with anthrax to recuperate their financial loss, which creates a possibility for anthrax transmission from animal to humans.

As a part of control and prevention activities of the government, Department of Livestock Services (DLS) is doing annual animal immunization routinely in the endemic districts and conducts the awareness campaign for boosting knowledge of cattle keepers and general people to some extent. Since the lack of sufficient paravets and logistics for animal vaccination at the Upazila level and the absence of a dedicated program on anthrax, the prevention and control program has faced challenges. However, due to the lack of proper awareness (100%) in the community level, the outbreak may happen in the same Upazila with changing the location (anon/personal collection). We present in this paper the findings of an epidemiological investigation of anthrax outbreaks that took place at the human-animal interface in the remote areas of Bangladesh. The purpose of

this study was to survey the outbreak situation in terms of animal, place, and time and to know the level of knowledge of people at the outbreak areas towards minimizing the risk of animal infection and subsequent spillover in human.

Materials and Methods

Outbreak details and investigation

Two animal anthrax outbreaks in Sirajganj district (Kamrakand and Shahjadpur Upazila) and one outbreak in Tangail district (Gopalpur Upazila) during April–June 2016 along with a single outbreak in Rajbari district (Kalukhali Upazila) in August 2017 were confirmed in cattle and investigated by the outbreak investigation teams (Fig. 1).

DLS received information from the field office that animals were suddenly ill with fever since May 13, 2016 in Jamtail village of Kamrakand Upazila of Sirajganj district. The DLS team investigated the outbreak on May 26, 2016 and documented that a bull on May 13, 2016 and a cow on May 24, 2016 were reported moribund with a high fever. Both animals were reported to be slaughtered, dressed, and sold in the community in <24 h of illness onset. The meat was sold in the village at the lower price (BDT 250/kg) and 12 people had skin lesions, who involved directly in the slaughtering of ill animal and

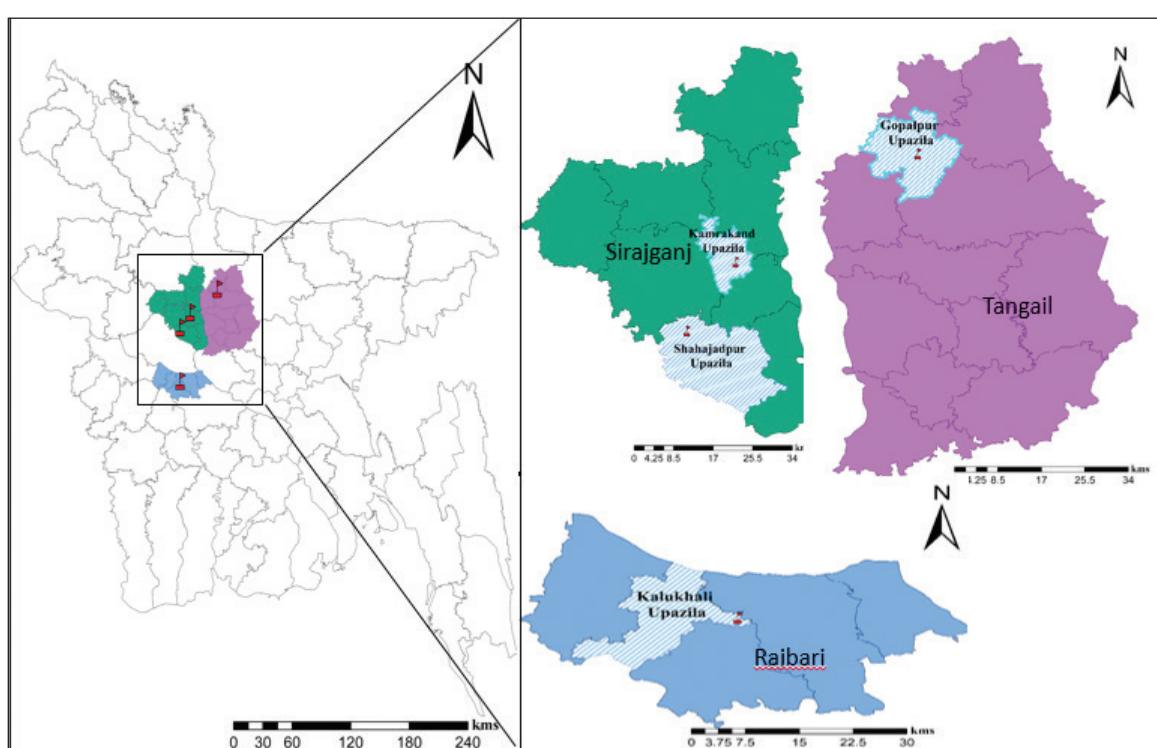


Figure 1. Map showing four anthrax outbreak investigation sites (Sirajganj-2, Tangail-1, Rajbari-1) during 2016-17.

processing of raw meat in their household. The team follow-up the event on June 1, 2016 and documented a similar animal and human illness history in the same village of Kamrakand Upazila: a bull was fallen down suddenly in a Hindu community, sold immediately to the Muslim neighbors. The ill bull was slaughtered and dressed and the meat was sold at a lower price. Nine people had got skin lesions who involved with animal slaughtering and meat processing. A total of three animals (two bulls and one cow) got sick and slaughtered in the same village within 1/2 km radius of Jamail cluster.

The DLS team was informed of an additional human anthrax outbreak in Bholarhat village of Tangail district on June 15, 2016 and North Mosipur village of Shahajadpur Upazila of Sirajganj district on July 10, 2016 after slaughtering of single ill cow in each of the villages with a history similar to anthrax in Jamail village of Kamarkand Upazila in Sirajganj district. The outbreak sites were investigated on June 16, 2016 and July 11, 2016, respectively. The investigation teams documented that a total of 23 people in Bholarhat village of Gopalpur Upazila of Tangail district and nine people in North Mosipur village of Shahajadpur Upazila of Sirajganj district had got skin lesions who involved actively in animal slaughtering, butchering, and meat processing. All human illness cases were confirmed as cutaneous anthrax and treated by the respective Upazila Health offices.

The DLS team was also informed through the local livestock office on a fresh anthrax outbreak in Sreerampur village of Kalukhali Upazila of Rajbari district on August 26, 2017 after slaughtering of an ill cross-breed cow with a history of bloody diarrhea and fallen down. The outbreak was investigated on September 8, 2017. The investigation team documented that a total of 17 people had got skin lesions who involved actively in animal slaughtering, butchering, and meat processing. All human illness cases were confirmed as cutaneous anthrax and treated by the Upazila Health Office.

An investigation team consisting of lab scientist, epidemiologist, and field veterinarian from the DLS visited the outbreak sites to explore the event in terms of place, time, and animal; that will help in strengthening the control program in future. Refrigerated meat samples of infected slaughtered animals were collected to confirm the causal agent. Participatory epidemiology tool such as semi-structured interview (SSI) was used in these investigations to assess the level of knowledge and practice of local people on anthrax control and prevention in animal and human as well.

An animal case was suspected for anthrax if the animal had a history of sudden death and draining of unclotted blood from natural openings. Alternatively, through the trace backing, an animal case of anthrax was confirmed if

the clotted blood of refrigerated meat of infected slaughtered animal contains rod-shaped bacilli that were found using the polychrome methylene blue (PMB) staining technique under the microscope with high power objective ($\times 100$) using immersion oil [1].

Data collection, management, and for statistical analysis

Data for both animal and human cases were captured and recorded separately in Excel worksheets and descriptive statistics were accomplished using Microsoft Excel® tools.

Results

Animal anthrax

Six animals (two bulls and four cows) were reported sick and documented in the three outbreak events. The median age of cattle was 27 months (range: 18–48 months). All (100%) cattle were slaughtered in <24 h of illness onset. The most common clinical history was high temperature, falling down, and lack of appetite (Table 1). The blood of the slaughtering animal was found to be unclotted was confirmed by the cattle farmers. The owners of the animal did not confirm anthrax vaccination in animal since in two cases (Shahajadpur and Gopalpur), the owner bought the new animal without anthrax vaccination history.

Human cutaneous anthrax

A total of 70 people had skin lesions in the three outbreak events: 21 in the first outbreak, 23 in the second outbreak and nine in the third outbreak and 17 in the fourth outbreak (Fig. 6).

Of reported people, 40 (57.4%) were male and 30 people (42.86%) were from 21 to 30 year age group. The mean period from contact with infection to illness onset was 3.5 days. All people had skin lesions, characterized by itching, fever, weakness, nausea, headache, and abdominal pain. The skin lesion was characterized by the existence of vesicle, central black eschar, and surrounding oedema. The skin lesions were frequently distributed in the finger (46.8%), lower arm (34%), upper arm (16%), and back (3.2%) (Table 1 and Fig. 2). All of the identified human cutaneous anthrax cases (100%) had a common history of slaughtering and dressing cattle and/or handled raw meat at the household or slaughtering site. Fifty-four percent of human cases had an exposure history of slaughtering and dressing of ill animal whereas 48% had a history of handling and processing raw meat.

Microscopic examination

The clotted blood from the refrigerated meat samples was tested with PMB staining technique that found rod-shaped bacilli (Fig. 3).

Table 1. Characteristics of six animal anthrax cases in Bangladesh, April–June 2016 and July 2017.

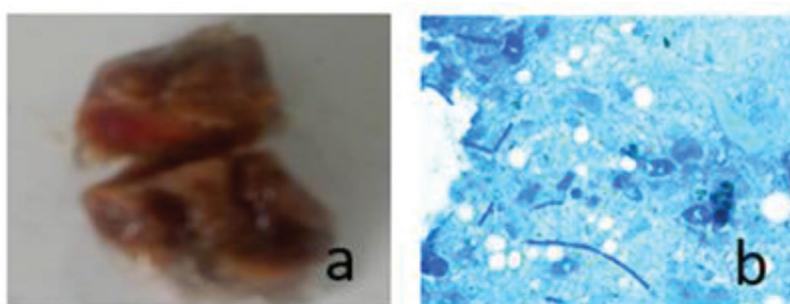
Characteristics	Jamtail village of Kamarkanda Upazila, Sirajganj district		Bholarhat village of Gopalpur Upazila, Tangail district		North Mosipur village of Shahjadpur Upazila, Sirajganj district		Sreerampur Village of Kalukhali Upazila, Rajbari district		Total	
Animal	n	%	n	%	n	%	n	%	N	%
Cattle	3	-	1	-	1	-	1	-	6	100
Age in months (median)	36	-	24	-	30	-	24	-	27	-
Clinical characteristics										
Fever	3	100.0	1	100.0	1	100.0	1	100.0	6	100.0
Fallen down	3	100.0	1	100.0	1	100.0	1	100.0	6	100.0
Lack of appetite	3	100.0	1	100.0	1	100.0	1	100.0	6	100.0
Diarrhea (bloody)	2	66.7	1	100.0	0	0.0	1	100.0	3	50.0
Muscle tremor	2	66.7	1	100.0	1	100.0	0	0.0	4	67.0
Respiratory distress	2	66.7	0	0.0	0	0.0	1	100.0	2	33.0
Outcome of sick animal										
Slaughtered	3	100.0	1	100.0	1	100.0	1	100.0	6	100.0
Vaccination history										
Anthrax	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Foot and Mouth Disease	3	100.0	1	100.0	1	100.0	0	0.0	5	83.3
Laboratory testing										
Positive in PMB microscopic examination	3	100.0	1	100.0	1	100.0	1	100.0	6	100.0



Figure 2. Cutaneous anthrax in (a) backside of the body (slaughtered and dressed ill animal), (b) base of the finger (handled and processed raw meat) at Jamtail village of Kamarkand Upazila of Sirajganj district, (c) in finger (slaughtered and dressed ill animal) and (d) arm (slaughtered and dressed ill animal) at Bholarhat village of Gopalpur Upazila of Tangail district (e) in finger (slaughtered and dressed ill animal) and (f) arm (handled raw meat) North Mosipur village of Shahjadpur Upazila of Sirajganj district (g) in arm (slaughtered and dressed ill animal) and (h) finger (slaughtered and dressed ill animal) at Sreerampur village of Kalukhali Upazila of Rajbari district were found during outbreak investigations.

Table 2. Characteristics of 70 human cutaneous anthrax cases in Bangladesh, April–June 2016 and July 2017.

Characteristics	Jamtail village of Kamarkanda Upazila, Sirajganj district		Bholarhat village of Gopalpur Upazila, Tangail district		North Mosipur village of Shahjadpur Upazila, Sirajganj district		Sreerampur Village of Kalukhali, Upazila, Rajbari district		Total	
Human	n	%	n	%	n	%	n	%	N	%
Age group (years)										
11–20	2	9.5	4	17.4	4	44.4	5	29.4	15	18.9
21–30	12	57.2	14	60.9	4	44.4	6	35.3	36	56.6
21–40	7	33.3	5	21.7	1	11.2	6	35.3	19	24.5
Sex										
Male	11	52.4	11	47.8	6	66.7	12	70.58	40	57.14
Female	10	47.6	12	52.2	3	33.3	5	29.41	30	42.86
Clinical expression										
Skin lesion	21	100	23	100	9	100	17	100	70	100.00
Itching skin	15	71.4	13	56.5	5	55.6	17	100	50	71.43
Fever	10	47.6	9	39.1	1	11.1	10	58.8	30	42.86
Headache	8	38.1	8	34.8	4	44.4	7	41.2	27	38.57
Nausea	7	33.3	2	8.7	1	11.1	3	17.6	13	18.57
Abdominal pain	1	4.8	2	8.7	0	0	0	0	3	4.29
Diarrhea	2	9.5	0	0	0	0	0	0	2	2.86
Distribution of skin lesion										
Back	2	6.3	1	3.4	0	0	0	0.0	3	3.2
Upper arm	3	9.4	7	24.1	1	11.1	4	16.7	15	16.0
Lower arm	12	37.5	9	31.0	3	33.3	8	33.3	32	34.0
Finger	15	46.9	12	41.4	5	55.6	12	50.0	44	46.8
Exposure via										
Slaughtered and dressed ill animal	13	61.9	10	43.5	5	55.6	10	58.8	38	54.3
Handled and processed raw meat	8	38.1	13	56.5	4	44.4	7	41.2	32	45.7

**Figure 3.** Microscopic examination of blood clots for *B. anthracis*. (a) Blood clots in the refrigerated meat samples; (b) rod-shaped bacilli using PMB staining technique from the meat sample of infected cattle of North Mosipur village of Sirajganj district.

Assessment knowledge and practices on anthrax prevention and control through SSI (animal owners and villagers)

A total of 100 peoples, who reared livestock for a livelihood, were interviewed in four outbreaks areas on anthrax awareness and as well as anthrax prevention and control practices, using SSI technique. Almost all of the people (93%) had no knowledge on anthrax; however, 40% reported that they heard the name Anthrax or Torka (Bangla name) earlier. Of the 100 people, only 25% people were informed

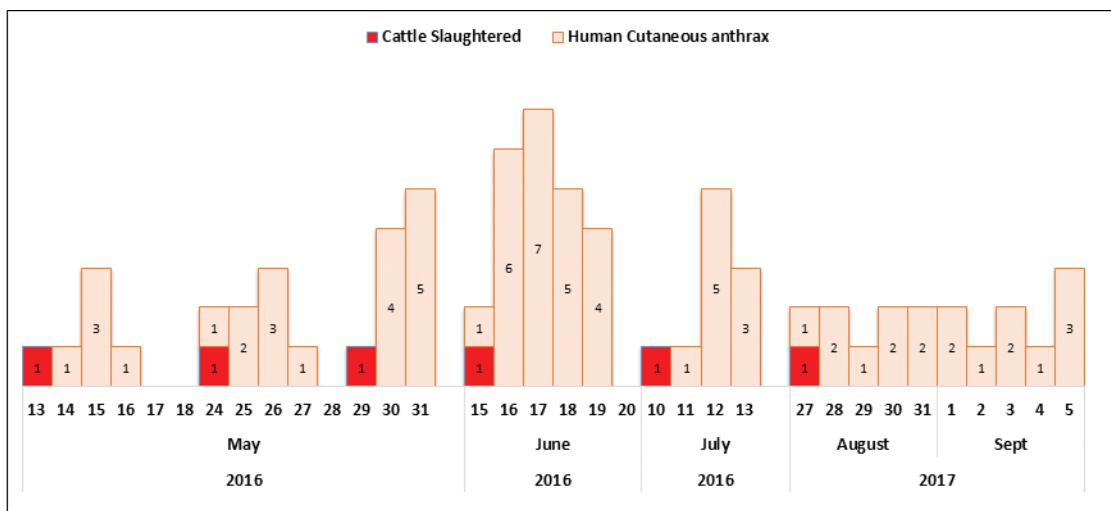
that vaccine can protect animals from anthrax infection. Simultaneously, people were asked about their meat consumption and dead animal disposal/burial practices. Ninety-two percent people reported that they consumed sick animal meat while no people consumed dead animal meat (Table 3).

Discussion

All anthrax-infected cattle were slaughtered and consumed by the community people. Since, all human cases have had

Table 3. Knowledge and practices on anthrax control and prevention in outbreak areas of three districts (Sirajganj, Tangail, Rajbari).

Parameter	Yes (%)	No (%)	95% CI
Knowledge			
What is Anthrax or Torka (Bangla name)?	7 (7%)	93 (93%)	2.9%–13.9%
Do you hear the name Anthrax or Torka (Bangla name)?	40 (40%)	60 (60%)	30.3%–50.3%
Vaccine can protect animal from anthrax?	25 (25%)	75 (75%)	16.9%–34.7%
Disease can be transmitted from animal to human during the slaughtering/dressing of an infected animal	2 (2%)	98 (98%)	0.2%–7.0%
Practice			
Consume sick animal meat	92 (92%)	8 (8%)	84.8%–96.5%
Consume dead animal meat	0 (0%)	100 (100%)	0%–3.6%
Disposal of dead animal in the open land or water bodies	67 (75%)	23 (25%)	56.9%–76.1%

**Figure 4.** Human cutaneous anthrax in relation with cattle slaughtered/meat processing/handle or dress raw meat was captured during outbreak investigation.

an epidemiological link with animal slaughtering, meat processing/handling activities; the finding is in compliance with other researchers in Bangladesh [5,9]. They established the association for the development of human cutaneous through involvement in animal slaughtering or dressing/washing of cattle/goat or their meat, handling raw meat, and contact with the animal skin. The temporal sequence of animal and cutaneous form of human anthrax indicates that animal anthrax is followed by human cutaneous anthrax; hence, supporting the theory that anthrax transmission occurs from animal to humans [3,9]. The reported outbreaks were started at the beginning of the monsoon when pasture land is adequate for grazing of cattle and this facilitates the opportunity of ingestion of vegetative form of anthrax spore and continue up until to the late monsoon season [10]. Few ecological parameters such as soil type, soil contents viz. calcium and organic carbon and soil pH play the important role for surviving of anthrax spore in outbreak-prone districts of Bangladesh as depicted by Ahsan et al. [11]. Alongside, lack of awareness and some risky behavior of farmers such as throwing the dead body of animals in water or in the open

land make an avenue for anthrax bacilli spillover to both animals and humans [3,10]. For these reasons, the recurrent outbreak occurs every year in these districts in both animal and human. To minimize this spillover risk in human, thus, a targeted vaccination strategy with a provision with twice a year could be opted out in anthrax risk-prone districts of Bangladesh with maximum vaccination coverage necessitated [10]. At present, DLS is producing around 6.00 million Stern spore vaccines for animal immunization [12] which could be sufficient for targeted vaccination in the high-risk districts. However, a single dose Stern spore vaccine could not be maintained with protective immunity level for a year until the introduction of an additional single dose as a booster [13–15]. On the contrary, the locally produced vaccine using Sterne F-24 stain of *B. anthracis* imported from Australia [16] can protect the vaccinated animal up to 1 year [17]. Uncontrolled animal movement and the influx of new animal in a vaccinated area induce further burden of fresh outbreaks. Animal vaccination card to be ensured for the cattle traders during animal movement towards friendly finding out the unvaccinated animal in the risky endemic districts.

In Bangladesh, livestock owner prefers to slaughter the sick animal to lessen their economic loss and this may cause human illness (i.e., skin lesions) mainly due to contact of human skin bruise with infected animal blood during animal slaughtering and meat processing. Considering this point, financial incentives could be provided for the animal owners as compensation for their financial loss. It has been accepted that cutaneous anthrax in human preceded after the animal anthrax and all recent animal anthrax outbreaks were identified through the backward-looking of the human cutaneous anthrax cases. Lack of the substantial level of public awareness in the outbreak areas is considered to be responsible for the human spillover [10,17].

The Global Health Security Agenda(GHSA) implies a multi-sectoral, One Health (OH) approach to be adopted towards strengthening both the global and nations' capacity to prevent, detect, and respond to human and animal infectious disease threats either naturally occurring or accidentally or intentionally, spread of a disease of zoonotic importance [18]. An approach to be installed for educating the local community on anthrax transmission and prevention, control phases such as real-time surveillance, animal vaccination including mass awareness creation and motivation that will help and thereby facilitate early detection, control, and prevention of anthrax outbreaks [5]. The anthrax control and prevention program could be conducted in all identified animal anthrax outbreak areas by the local livestock and health officials. Safe disposal of dead animals, best slaughtering practices, and safe handling of cattle meat are important considering public health and food safety issues.

Conclusion

A comprehensive preparedness and response guidelines (strategies) should be formulated including the activities relating to surveillance, communication, vaccination, outbreak investigation and to be approved by the government; and implemented in the high risky districts towards minimizing animal anthrax at source is demanded. These activities will help to reduce the further spillover in human by motivating cattle owners and villagers through the mass awareness creation under OH approach.

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Conflict of interests

The authors declare no conflict of interest.

Authors' contribution

The study designed and conducted through active participation in the area of outbreak investigation, and debriefed on prevention and control strategies and legal aspects to the community people by SK Shaheenur Islam, Golam Azam Chowdhury, Shovon Chakma, Nelima Ibrahim, Faisol Talukder and collected samples for laboratory testing and performed PMB microscopic examination in the laboratory by Golam Azam Chowdhury; and SK Shaheenur Islam, Shovon Chakma, and A. H. M. Taslima Akhter conducted literature review, statistical analysis, and wrote the manuscript and Shovon Chakma prepared map using GIS. Line listing Human sickness cases was prepared by Faisol Talukder during the outbreak investigation. All authors read and approved the final manuscript unanimously.

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