OPEN ACCESS

J. Adv. Vet. Anim. Res., 2(2): 158-164.
Available at- *http://bdvets.org/JAVAR*

JAVAR Journal of Advanced Veterinary and Animal Research

ORIGINAL ARTICLE

Volume 2 Issue 2 (June 2015)

DOI: 10.5455/javar.2015.b72

Factors associated with repeated outbreak of anthrax in Bangladesh: qualitative and quantitative study

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ABSTRACT

Anthrax, caused by *Bacillus anthracis* is an acute, febrile disease of warm blooded animals including humans. Social norms and poverty in addition to climatic factors such as soil conditions, seasons of year, ambient temperature and rainfall influence the persistence of the *B. anthracis* and anthrax outbreaks. The present study was designed to reveal the factors influencing the repeated outbreak of anthrax in Bangladesh. Considering the previous outbreaks of anthrax, Sirajganj, Bogra, Kushtia, Tangail and Mymensingh districts of Bangladesh were selected for this study. To elucidate the factors, qualitative data relating to the animal management, knowledge and behavior of the people; and quantitative data relating to soil conditions, ambient temperature and rainfall were acquired, and analyzed critically. Based on the outbreak histories, a year was divided into two seasons, anthrax prone season (May-November) and anthrax dry season (December-April). Anthrax spores could be isolated from 11.67% (n=14/120) of the soil samples collected from the study areas. The present study revealed that poor knowledge, lack of awareness, improper carcass disposal, inadequate vaccination, high Ca content and moisture in the soil along with high ambient temperature and rainfall during the anthrax prone season were the possible influencing factors of repeated outbreaks of anthrax in the study areas. Intensive propaganda to create public awareness of anthrax together with proper vaccination may reduce anthrax outbreaks in Bangladesh.

Keywords

Anthrax prone season, Knowledge, Managerial factor, Rainfall, Repeated outbreak, Soil, Temperature

ARTICLE HISTORY

Received : 12 November 2014, **Revised**: 3 January 2015,
Accepted : 5 January 2015, **Published online**: 3 February 2015.

INTRODUCTION

Anthrax (popularly known as "*Torka*" in Bangladesh) is an acute infectious zoonotic disease caused by *Bacillus anthracis*, a soil-borne, spore forming bacterium (OIE, 2008). The anthrax spore (*i.e.*, dormant stage) is resistant to heat and chemical disinfectants, and this dormant stage may persist and remain viable for several decades in soil (Hirsh and Zee, 1999; Dragon et al., 2001; OIE, 2004). The bacterium primarily infects herbivores such as cattle, sheep, goats, horses and pigs after entering the body through feed and water contaminated with viable spores (Ahsan et al., 2013). Anthrax is transmitted from the affected animals to humans through food or other material originated from an animal that is contaminated with *B. anthracis* or its spores (Cieslak and Eitzen, 1999; WHO, 2008).

Anthrax naturally occurs in almost all countries in the world; however, the disease is mostly prevalent in tropical and sub-tropical countries (Biswas et al., 2011). In recent years, the disease has been reported in Sweden (Lewerin et al., 2010), USA (Mongoh et al., 2008), Italy (Fasanella et al., 2010), Australia (Durrheim

et al., 2009), and many places in Europe. In many Asian and African countries, anthrax outbreak occurs periodically in animals and humans (WHO, 2008). In Bangladesh, the disease was found periodically in animals and humans until 2009 (Ahmed et al., 2010), but in recent years the disease has occurred repeatedly; the outbreaks indicate that the disease is no longer sporadic rather than enzootic in Bangladesh (Ahmed et al., 2010; Fasanella et al., 2012; Ahsan et al., 2013).

In Bangladesh, anthrax outbreak is mostly prevalent in Sirajganj and nearby districts (Ahmed et al., 2010; Biswas et al., 2011). Along with animal infections, more than 600 people have been diagnosed with anthrax in Bangladesh until the year 2011 (Fasanella et al., 2012). Inadequate livestock vaccination coverage, butchering sick animals, disposing butchering wastes and carcass in the environment where animals graze, handling raw meat, contact with sick animals, social norms, and poverty contributed to the outbreaks of anthrax in Bangladesh (Chakraborty et al., 2012; Islam et al., 2013). Favorable environmental conditions such as, soil pH, Ca content, moisture, soil type, high ambient temperature and rainfall and topography are positively correlated with the persistence of anthrax spores and subsequent outbreaks (Van Ness, 1971; Hugh-Jones, 2002; Ahsan et al., 2013). In our previous study (Ahsan et al., 2013), we investigated the environmental factors related to repeated anthrax outbreak in Bangladesh. Though there is discrete information regarding the social norms, poverty and ignorance, there is no comprehensive data on the knowledge and behavior of the people, environmental parameters (soil factors and ambient temperature and rainfall) that might positively influence on the outbreaks of anthrax. Here, our investigation focused on the present status of knowledge and behavior of people along with the management of livestock and related environmental parameters in order to understand how they might influence the repeated anthrax outbreaks in Bangladesh.

MATERIALS AND METHODS

Selection of study area and study population: The study areas were selected by assessing the risk of anthrax and from reports published in daily newspapers. The study was conducted in Shahjadpur and Ullahpara Upazila of Sirajganj, Sadar Upazila of Bogra, Veramara Upazila of Kushtia, Sadar Upazila of Tangail and Mymensingh districts. Each time, the study team visited the reported areas and collected information and susceptible samples for subsequent analysis. To ascertain the outbreak and outbreak areas, the study team firstly visited the human and livestock officials of the particular areas. The animal owners and their family members, people associated with slaughtering, butchering, handling and eating the meat of affected animals, people who developed lesions of cutaneous anthrax, people who were neither associated with processing of carcass nor had developed lesions, the community leader, livestock and health officials of the outbreak areas were considered as the key informants.

Environmental factors associated with anthrax outbreak: The environmental factors such as soil type, moisture, pH, Ca content and organic carbon contents were determined following continuous collection and examination of the soil samples (n=120) from the study areas throughout the year. Approximately 400-gm of surface soil from a maximum depth of one-foot was collected according to the procedure mentioned by Ahsan et al. (2013). Soil samples were collected from the areas where the suspected animals died, slaughtered and butchered or buried. One blood sample (5 mL in a disposable plastic syringe) from the jugular vein of affected animal and four swab samples from the ulcerative lesions of affected human were also collected. The swab samples were immediately dipped in screw capped vial containing nutrient broth. The samples were carried to the laboratory in ice box within possible earliest period. Physico-chemical analysis of the soil samples were performed in the Soil Science Laboratory at the Department of Soil Science, Faculty of Agriculture, Bangladesh Agricultural University. The changes in the soil parameters with time were determined and recorded accordingly. The weather related data (ambient temperature and rainfall) of the study districts were collected from the official website of world weather (http://www.worldweather online.com and http://www.accuweather.com). According to the history of outbreak, anthrax outbreaks in Bangladesh mostly occurred during the months of May to November. Based on the histories, a year was divided into two seasons, anthrax prone season (May to November) and anthrax dry season (December to April). Data derived from the soil sample analysis, ambient temperature and rainfall were processed and analyzed based on the seasons mentioned.

Host and managerial factors associated with anthrax outbreak: Some tools of qualitative research were used

with the aim of unfolding the possible causes of repeated anthrax outbreak or transmission to human in the areas relating to the knowledge, behavior and managerial factors (*i.e.*, feeding, vaccination, treatment etc.). Data related to host and managerial factors were collected from the people interviewed in the study area. Qualitative research tools used in this study were- (a) Direct Observation, (b) Focus Group Discussion, and (c) In-depth interview of key informants.

Data analysis: All the data obtained from the study were analyzed critically to discover the factors possibly triggering the repeated outbreak of anthrax in Bangladesh. The analyses were done using Statistical Package for Social Science (SPSS) version 17.0 for windows (Chicago: SPSS Inc.). Chi-Square test (test for independence or relatedness) was used to determine the significant relationship among the qualitative variables and quantitative data were analyzed using One Way Anova. A p-value ≤ 0.05 was considered as significant.

RESULTS AND DISCUSSION

Anthrax is now an emerged enzootic in Bangladesh (Ahmed et al., 2010). This study covered those areas in Bangladesh where anthrax outbreak occurred annually both in animals and human beings. Knowledge and behavior of the people in relation to anthrax along with the animal management, soil conditions, ambient temperature, and rainfall were studied to elucidate their influence on the repeated outbreaks of anthrax in Bangladesh.

The study revealed that the primary outbreaks were confined to rural and very remote areas in Bangladesh where most of the people are not well informed about diseases of animals and the transmission of animal diseases to humans. In the affected areas, 56.67% (n=153/270) people had no knowledge about anthrax disease, its outbreak and the consequences (**Table 1**). Among the people who had knowledge of anthrax, 50.83% people did not have knowledge on the proper process of carcass disposal (**Figure 1**). In most cases (79.00%), they left the carcasses in open fields or floating in nearby river water (**Figure 2**). The close proximity of the farms to the river, and flooding were positive contributory factors to the outbreak of anthrax (Mongoh et al., 2008; Lewerin et al., 2010). The surrounding areas of the anthrax outbreak households of Sirajganj and Bogra districts were in low land areas; flood prone and beside the basin of river. This study also isolated anthrax spores from the soils from those areas. In our study, we found that 81.25%, 81.25% and 63.64% people at Ullahpara, Shahjadpur and Bogra sadar upazila fed their animals unwashed grass (**Figure 3**). Among the people who washed grass before feeding to their animals, most (75.71%) used river water (**Figure 4**); thus possibly making the animals vulnerable to anthrax.

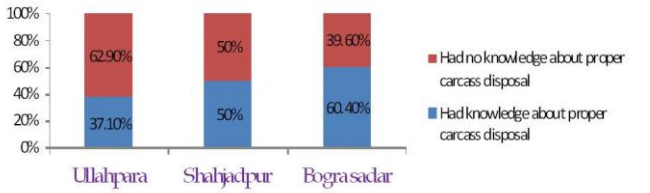

Figure 1. Knowledge of the respondents of different areas about proper disposal of anthrax affected carcass.

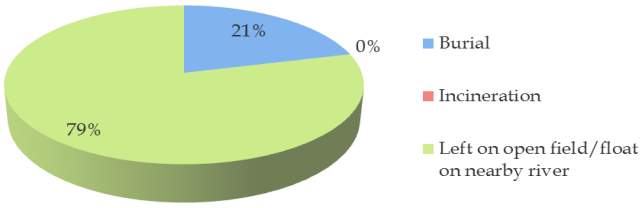

Figure 2. Methods of carcass disposal usually followed by the respondents.

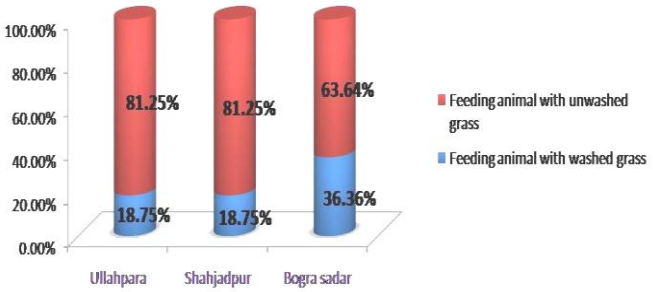

Figure 3. Washing of grasses before supplying to the animal.

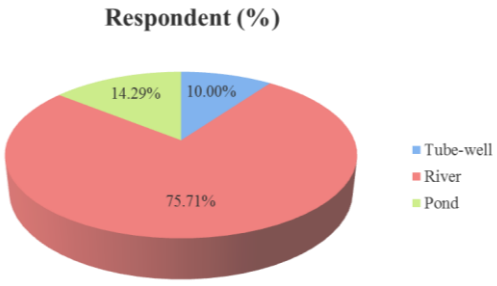

Figure 4. Sources of water used for washing grasses.

Table 1. Level of knowledge of the respondents about anthrax (N=270).

Place	Level of knowledge about anthrax				
	Good	Moderate	Poor	None	*p*-value (χ^2 test)
Ullahpara	7 (8.75%)	7 (8.75%)	7 (8.75%)	59 (73.75%)	
Shahjadpur	5 (6.25%)	10 (12.5%)	25 (31.25%)	40 (50%)	**0.000****
Bogra Sadar	16 (14.55%)	24 (21.81%)	16 (14.55%)	54 (49.09%)	
Total	**28 (10.37%)**	**41 (15.19%)**	**48 (17.78%)**	**153 (56.67%)**	

Table 2. Physico-chemical properties of anthrax positive and negative soil samples.

Parameters	Positive Samples (Mean±SE)	Negative Samples (Mean±SE)	*p*-value (One-way Anova)
Moisture (%)	16.69±2.06	20.24±1.30	0.292
pH	6.38±0.15	6.17±0.05	0.132
Ca (PPM)	831.77±62.16	631.91±44.80	0.083
Organic Carbon (%)	0.86±0.17	0.95±0.06	0.556

Table 3. Physico-chemical properties of samples collected during anthrax prone (May – November/2012) and dry seasons (December/2012-April/2013).

Parameters	Anthrax Prone Season (Mean±SE)	Anthrax Dry Season (Mean±SE)	*p*-value (One-way Anova)
Moisture (%)	15.18±1.25	24.47±1.77	**0.000****
pH	6.13±0.08	6.27±0.050	0.137
Ca (PPM)	927.91±45.35	358.65±30.45	**0.000****
Organic Carbon (%)	1.00±0.08	0.85±0.07	0.178

Table 4. Temperature and rainfall during the anthrax prone (May-November, 2012) and dry season (December, 2012-April, 2013).

Parameter	Anthrax Prone season (Mean±SE)	Anthrax Dry season (Mean±SE)	*p*-value (One-way Anova)
Average Temperature	29±0.458	23.48±1.033	**0.000****
Rainfall	185.60±25.974	22.24±5.675	**0.000****

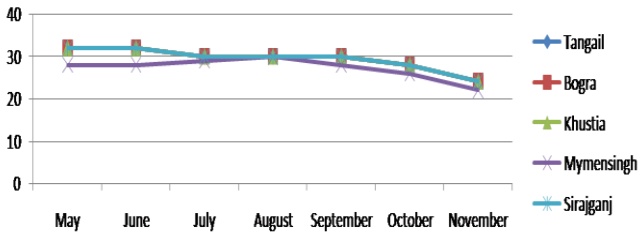

Figure 5. Average temperature (°C) recorded during the anthrax prone season (May-Nov, 2012).

Environmental parameters (soil conditions, ambient temperature, and rainfall) are potential associates of spore survival and ecological conditions of repeated outbreak of anthrax. Soil type, moisture, pH, Ca and organic carbon content strongly influence the length of survival of anthrax spores in the soil (Ahsan et al., 2013). According to previous history, anthrax outbreak in Bangladesh usually occur during the period from late April or May to November. In this study, based on the probability of anthrax outbreak, a year was divided into two seasons, *viz*. anthrax prone season (May to November/2012) and anthrax dry season (December/2012 to April/2013). Soil samples were collected from different study locations, and were examined for physicochemical and microbiological properties. Within 120 soil samples, 70 were collected from the anthrax prone period, and 50 samples were collected during dry season. Upon staining and microbiological examination, 11.67% (n=14/120) of the samples were found to be positive for *B. anthracis* spores, and all the positive isolates were isolated from loamy type soils collected during anthrax prone seasons, as reported by Ahsan et al. (2013). However, Ahsan et al. (2013) found anthrax spores in 29.17% (n=14/48) of the samples; this variation might be due to variation in sample size or location of sample collection. Physicochemical analyses of the samples were done for their moisture, pH, Ca and organic carbon contents, and the data were analyzed based on the presence or absence of *B. anthracis* and season in which they were taken. According to our analysis, there was no significant difference between anthrax positive and negative samples in regards to their moisture, pH, Calcium and organic carbon contents (**Table 2**). However, moisture and Ca content in soil samples of anthrax prone season differed significantly from that of dry season (**Table 3**). This finding was close agreement with the reports of Fox et al. (1973) and Moazeni et al. (2004).

Heavy rainfall after a long dry period has been found to influence the outbreak of anthrax (Moazeni et al., 2004). Van Ness (1971) reported that ambient temperature in excess of 15.5°C provided a microenvironment that promotes cycling of anthrax spores. In 2012, anthrax outbreak occurred in Ullahpara and Belkuchi of Sirajganj district in the month of May; in Shahjadpur of Sirajganj district, Bheramara of Kushtia district, Goalpara of Tangail district and Bogra sadar of Bogra district in the month of June and in Gangni and Mujibnagar of Meherpur district in the month of August (IEDCR, 2012). All of these anthrax outbreaks of 2012 occurred in summer season and the

average temperature of these areas was 29±0.458°C. In the anthrax dry season (December, 2012-April, 2013), the average temperature of the study areas was 23.48±1.033°C. The difference between the average temperature of anthrax prone season (May, 2012-November, 2012) and anthrax dry season was highly significant (**Figure 5-6**; **Table 4**). The average rainfall was also significantly different between anthrax prone and dry seasons (**Figure 7-8**; **Table 4**). Thus, high temperature with heavy rainfall in the anthrax prone season may contribute to the anthrax outbreaks.

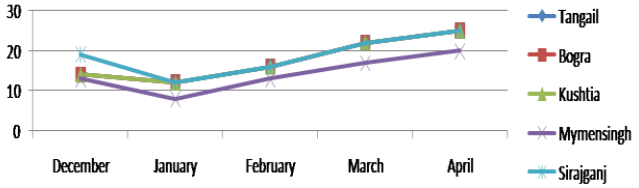

Figure 6. Average temperature (°C) recorded during the anthrax dry season (Dec, 2012-April, 2013).

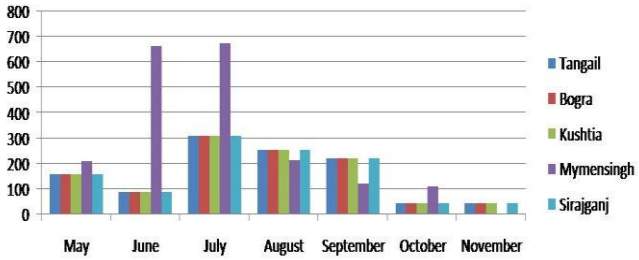

Figure 7. Average rainfall (mm) recorded during the anthrax prone season (May-Nov, 2012).

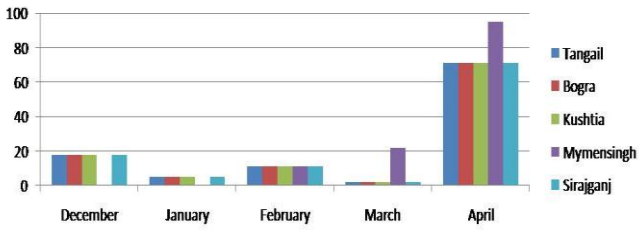

Figure 8. Average rainfall (mm) recorded during the anthrax dry season (December, 2012-April, 2013).

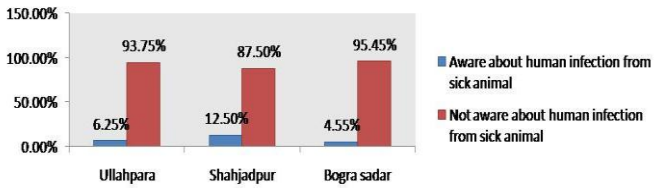

Figure 9. Awareness of the peoples of the study areas about human infection from sick animals.

Every previous human anthrax outbreak in Bangladesh occurred due to slaughtering of sick or moribund animals, presence at the slaughtering site and the handling of raw meat and meat products (Samad and Hoque, 1986; HSB, 2009). In our study, we identified that most of the people (93.75% in Ullahpara, 87.50% in Shahjadpur, and 95.45% in Bogra sadar) were not well informed about the potential transmission of infections from sick animals to humans (**Figure 9**). The knowledge of transmission of anthrax from animal to humans did not differ among the respondents from different location (p>0.05) but the overall knowledge of the respondents differed significantly (p<0.05).

For confirmatory diagnosis and discovering the source of outbreaks, it was crucial to isolate the organisms from suspected sources. Along with soil samples, blood and swab samples were confirmed to be positive for *B. anthracis*. Microbiological examinations were performed following the procedure described by "Manual for Laboratory Diagnosis of Anthrax" by CDC (2001), WHO (2003), OIE (2008) and Ahsan et al. (2013). However, due to limitations of laboratory facilities, we could not analyze water and grass samples collected from the study areas, and we also were not able to perform a molecular characterization of the isolated *B. anthracis*. Isolation of the organisms from different sources along with genetic relatedness studies would help us to address the origin of the outbreaks precisely.

Effective vaccination may be the best strategy to control anthrax in animals. Moreover, outbreak of anthrax in humans could be controlled if the animal anthrax outbreak were controlled successfully. This could be done by increasing public awareness of proper procedures to minimise transmission risk together with implementation of an effective vaccination program nationwide. Through national programs, there has been a progressive global reduction in animal anthrax cases over the past three decades. The disease is now absent or only sporadic in the West Europe and the North America (Ahmed et al., 2010).

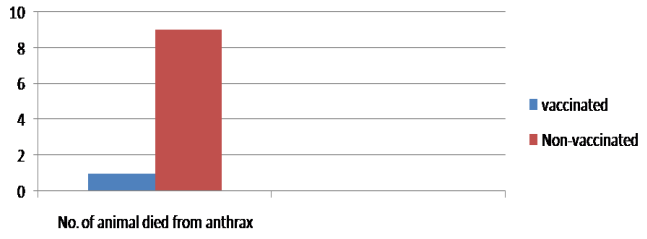

Figure 10. Vaccination History of the animals died from anthrax during the study period.

In our study, we found that animal deaths due to anthrax differed significantly (p<0.05) between the vaccinated and non-vaccinated groups. Among the 10 animals that died during the study period, 90% (n=9/10) were found to be non-vaccinated (**Figure 10**). This finding seems to indicate that vaccination is still an effective method to control anthrax outbreaks in Bangladesh. Acute shortage of Anthrax vaccine along with a shortage of veterinary facilities, including man-power, is responsible for the non-vaccination of animals. Deaths of animals even after vaccination may be due to vaccination failure. Immunity development upon vaccination largely depends on effective vaccine and proper vaccination technique (Chakraborty et al., 2012).

CONCLUSION

A complex interplay of lack of awareness, improper carcass disposal, poor management practices, high Ca and organic carbon content of the soils, high temperature and rainfall, and lack of vaccination are the possible causes of the repeated outbreak of Anthrax in Bangladesh. Ensuring public awareness and vaccination of the livestock population along with efficacy study of the anthrax vaccine produced by Livestock Research Institute (LRI), Bangladesh, will make a large contribution to the control of anthrax outbreaks in Bangladesh.

ACKNOWLEDGEMENT

We acknowledge the financial support from Bangladesh Agricultural Research Council (BARC) for this study.

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