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SHORT COMMUNICATION

ELISA Based Anthrax Antibody Titer in Cattle Induced by Locally Prepared Anthrax Vaccine Originated from Sterne F-24 Strain in Bangladesh

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ABSTRACT

Vaccination is usually practiced to prevent and control anthrax in Bangladesh. For this purpose, vaccine prepared from Sterne F-24 strain of*Bacillus anthracis*by Livestock Research Institute (LRI), Mohakhali, Dhakahas long been used in this country. However, in some cases anthrax occurred in vaccinated animals in Bangladesh. A total of 100 cattle at LalTeer Livestock Research and Development Farm, LalTeerLivestock Limited, Bangladesh, aging between 3-6 years and weighing between 250-400 kg were randomly selected for vaccination purpose. Blood samples (n=100) were collected before the vaccination for collecting pre-vaccination serum, andthe animals were vaccinated (at 1 mL/animal; $1x10^7$ spores/mL) with the anthrax vaccine produced by LRI. All blood samples from the vaccinated animals were collected on day 7, 28, 60, 90, 120, 150, 180, 240, 270, 300, 330, and 360 of post-vaccination, and serum samples were prepared. The antibody levels in the serum samples against anthrax were monitored using an Enzyme-Linked Immunosorbent Assay (ELISA). Over the course of 12 months, the antibody titers were found at the level higher than the reference value. Though there were reports on anthrax suspected cases in this farm, no such cases were reported during the study period. Thus, the vaccine appears to induce adequate antibody response against anthrax in Bangladesh.

Keywords: Cattle, ELISA, Antibody titer, Vaccine, Sterne F-24 strain, Bangladesh

Introduction

Anthrax (popularly known as "*Torka*" in Bangladesh) is an acute infectious disease caused by *Bacillus anthracis* (Weisss *et al*., 2007; OIE, 2008). Although the disease is found in almost all countries, it is mostlyprevalent in tropical and sub-tropical countries (Biswas *et al*., 2011). In recent years, the disease has been reported in Sweden (Lewerin *et al*., 2010), Italy (Fasanella *et al*., 2010), USA (Mongoh *et al*., 2008), Australia (Durrheim *et al*., 2009), and some other countries in Europe. *B. anthracis* forms spore in unfavorable conditions, which is resistant to heat and chemical disinfectants, and this may persist in soil as viable for several decades (Hirsh and Zee, 1999; Dragon *et al*., 2001; OIE, 2004).

Primarily, herbivores like cattle, sheep, goat, horse and pig are affected with anthrax (Ahsan *et al*., 2013) and the disease is usually fatal in ruminants (Islam *et al*., 2013).In humans, anthrax is almost invariably contract anthrax directly or indirectly from animals (WHO, 2008). In Bangladesh, the disease was sporadically reported in animals and human until 2009 (Ahmed *et al*., 2010). In recent years, the disease occurred repeatedly; the outbreaks indicated that the disease is no longer sporadic, rather enzootic in Bangladesh (Ahmed *et al*., 2010; Fasanella *et al*., 2013; Ahsan *et al*., 2013).In different areas of Bangladesh, 14 outbreaks occurred during the period of August 2009 to October 2010, affecting 140 animals and subsequently to 273 humans. This created a panic to the people. As a result in 2010, Bangladesh Government announced a 'Red Alert' throughout the country. Consequently, beef price and foreign exchange were decreased by 126% and 17% respectively, and about 35% small scale butchers were drove out of business in 2010 (Uddin, 2011). Along with social and environmental factor scarcity and limited vaccination was mentioned as one of the contributing factors of repeated outbreaks of anthrax in Bangladesh (Chakraborty *et al*., 2012; Islam *et al*., 2013; Hassan *et al*., 2015).

Vaccination is usually practiced in Bangladesh to prevent anthrax in animals. For this vaccination, Sterne F-24 stain of *B. anthracis* originated from Australia is used in Bangladesh (Roy *et al*., 2013). A single dose of vaccine is given to each animal that protects the animal for one year from anthrax. The protective effect of a single dose of strain 34F2 vaccine is said to last about one year (Sterne, 1939). However, Successful development of protective immunity against anthrax in animals requires effective vaccine and proper vaccination technique. Also, a single dose of Sterne vaccine may not be sufficient to ensure protective immunity in the animal to last for a year, and more than one initial dose of the Sterne vaccine may be necessary (Turnbull *et al*., 2004; WHO, 2008; Mongho *et al*., 2008). Very few studies focusing on immunological characterization have been conducted with cattle and goats, and the previous studies covered a limited period of time (Roy *et al*., 2013; Dipti *et al*., 2013; Nagarajan *et al*., 2015). Moreover, the previous studies have been conducted with a limited number of animals in experimental condition. Recently, we reported the causes of repeated anthrax outbreak in Bangladesh as well as the present status of knowledge, attitude, and behavior of people towards anthrax (Ahsan *et al*., 2013; Hassan *et al*., 2015). Here, we determined the titer of anthrax antibody level in cattle under commercial farm condition throughout the year using Enzyme Linked Immunosorbent Assay (ELISA).

Materials and Methods

The study was performed in a commercial animal facility at Lal Teer Livestock Research and Development Farm, Lal Teer Livestock Limited, Bangladesh, located at Bongao, Uthura, Valuka, Mymensingh, during the

period from April 2013 to April 2014. The anthrax vaccine was collected from the Livestock Research Institute (LRI), Mohakhali, Dhaka, Bangladesh. The master seed of this vaccine contained living spore of the non-capsulated attenuated Sterne F-24 strain of *B. anthracis* obtained originally from Australia. A total of 100 healthy and unvaccinated cattle at the farm were randomly selected for this study. The cattle received anthrax vaccine in previous years as a routine work. However, the cattle did not receive any anthrax vaccine within past one year when the study started. The cattle were aged between 3-6 years weighing between 250-400 kg.

For the determination of antibody level against anthrax, blood samples (10 mL each; n=100) were collected before vaccination of the animals. Then all the cattle were vaccinated following the schedule mentioned by LRI. Briefly, 1 mL vaccine containing 1×10^7 spores (approximately) was injected per animal through subcutaneous route at neck region. Repeat sets of blood samples (n=100) were collected from the vaccinated animals on day 7, 28, 60, 90, 120, 150, 180, 240, 270, 300, 330, and 360 of post-vaccination. All blood samples were carried in ice box to the Laboratory at the Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202. Serum samples were prepared from the blood samples according to the method described by Dipti *et al*. (2013). The antibody level in the serum samples were determined by ELISA (AniGen*B. anthracis*Ab ELISA kit, BIONOTE Inc., Republic of Korea).

Results and Discussion

The cattle vaccinated with local anthrax vaccine of Bangladesh were found to elicit sufficient immune response and production of antibody level above the reference value over a period of 12 months (**Table 1**; **Fig. 1**). According to the ELISA kit, samples to positive ratio (S/P ration)≥0.5 was indicative of vaccination or previous exposure to anthrax. The pre-vaccination S/P ratio obtained in this study was 0.16±0.05, indicated that the cattle had low level of antibody against anthrax, which could be produced during the previous year vaccination cycle. Vaccination triggered antibody production and was reached above the reference value at day 7 of post-vaccination (**Table 1**). The highest S/P ratio was recorded as 2.15±0.17 at day 28 of post-vaccination (**Fig. 1**). The antibody levels over the year were found fluctuating giving a minimum S/P ratio of 1.07±0.14 on day 180 of post-vaccination (**Table 1**).Though there were some reports of anthrax suspected cases in the study farm (personal communication), there was no reports of such cases during the study period, indicating that the vaccinated animals developed protective immunity against anthrax. However, due to the lack of experimental facilities we could not perform challenge experiment. Thus, it was not possible to determine the minimum protective antibody levels against anthrax infection induced by the local anthrax vaccine. Vaccination of animal with local anthrax vaccine followed by challenge exposure with virulent *B. anthracis* strains isolated from various host species would reveal the minimum protective antibody levels induced by the vaccine. Experimental evidence shows that antibody induced by Sterne strain could protect guinea pig against pathogenic *B. anthracis* isolated from different animals (Little and Knudson, 1986). In addition to the vaccine type, health status of animal is crucial to develop proper immune response (IOWA Beef Center website).

Vaccination response as obtained in this study differed from the finding of Dipti *et al*. (2013) and Roy *et al*. (2013) although they used same vaccine that we used in our study, who could detect early immune response in cattle at day 30 of immunization; whereas, in the present study we revealed early response at day 7 of post-vaccination. In addition, Dipti *et al*. (2013) described a steady increase in antibody level upto 90days. In contrast, we found the antibody level was fluctuating over the study period (**Fig. 1**). The fluctuating nature of antibody in the body might be due to being thevaccine as live spore vaccine, and health status of the responders. Live vaccines after introduction into the body goes under multiplication, like BCG vaccine goes under multiplication in the injection site and in the local draining lymph induces profound inflammatory and immune responses by activating T and B lymphocyte (WHO website). Anything that alters the replication of the organisms in the body can cause altered immune response induced by the vaccine as well as render the vaccine become ineffective (CDC website). However, our findings strengthen the findings of Dipti *et al*. (2013) and Roy *et al*. (2013) who conducted the research works up to 90 days. In this study we report the results for an extended period of time upto one year.

Table 1: S/P ratios of ELISA tests of serum samples collected from the cattle vaccinated with LRI produced Anthrax spore vaccine. Samples to positive ratio (S/P ration) ≥0.5 was indicative of vaccination or previous exposure to anthrax.

Serum collected at days of vaccination	S/P ratio (Mean±SE)
Day 0 (Pre-vaccination)	0.16±0.05
Day 7	1.31±0.17
Day 28	2.15±0.17
Day 60	1.34±0.16
Day 90	1.47±0.18
Day 120	1.08±0.18
Day 150	1.25±0.16
Day 180	1.07±0.14
Day 240	1.43±0.19
Day 270	1.53±0.12
Day 300	1.44±0.20
Day 330	1.80±0.18
Day 360	1.84±0.48

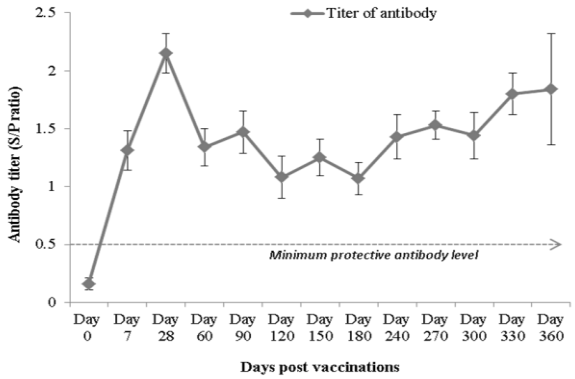

Figure 1. Antibody level of the vaccinated animals against anthrax. As per the instruction of the ELISA kit used in this study, 0.5 was considered as the minimum protective level (green arrow) against anthrax.

Conclusion

The anthrax vaccine manufactured by LRI, Mohakhali, Dhaka, Bangladesh could protect the vaccinated cattle from anthrax up to one year. Therefore, the anthrax vaccine produced by this institute may be effectively used to prevent anthrax outbreaks in animal population of Bangladesh. However, further study of the immunogenicity of the vaccine along with challenge exposure is recommended to establish whether, the antibody induced by the vaccine is protective or not.

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