Efficaciousness of Sterne 34F-2 strain of *Bacillus anthracis* vaccine in cattle for anthrax control program in Bangladesh

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**ABSTRACT**

Live spore Sterne 34F-2 strain of *Bacillus anthracis* vaccine is being used to control anthrax disease in Bangladesh. After produced this vaccine in Livestock Research Institute (LRI) under the Department of Livestock Services (DLS), it is distributed at the farmers level through district and Upazila Livestock Offices. In these distribution pathways, the vaccine has been transported and stored for a few days in each station. The present study was carried out to evaluate the humoral immune response of the anthrax vaccine and to measure the impact of existing transportation and storage systems on immunity status. For that a total of 60 cattle were randomly selected, divided into three groups and used the vaccines collected from three distribution points. Serum samples were collected before and after the 1st month, 4th month, 7th, 10th, and 13th month of vaccination respectively the anthrax antibody level in blood were monitored. The optical density was converted to ELISA units (EU) and used to express the antibody level in the vaccinated animals. It was significantly increased above the protection level (1.00) for a year. Before vaccination, the average ELISA unit of serum sample was 0.18± 0.01, after vaccination it was raised above the protective level (1.00) within one month and continued up to a year. In the chi-square test (95% confidence level), there was no significant difference (p<.05) ELISA unit among the three groups that means no impact on vaccine distribution points on the immunity level of the studied animal. The Sterne 34F-2 strain *Bacillus anthracis* vaccine has been found to be efficacious to protect animals from anthrax in the rural areas and no significant impact on immune response due to existing transportation and storage facilities.

**Keywords:** Sterne 34F-2, *Bacillus anthracis*, ELISA, IgG, community household cattle

**Introduction**

Among the various infectious diseases that cause a hundred to thousands of death of livestock animals in Bangladesh, Anthrax is one of them. This disease is caused by *Bacillus anthracis*; a spore-forming gram-positive bacterium that can survive in soil as dormant for decades (Saile & Koehler, 2006; Hugh-Jones & Blackburn, 2009; Silvestri et al., 2015). It is considered a serious disease of livestock because it usually strikes suddenly with livestock showing few signs of illness before dying and has zoonotic importance (Shiferaw, *Corresponding Author: K. H. M. Nazmul Hussain Nazir*

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For this study, a total of 60 cattle of due to lack of facilities or other adversity like ice melt C) and transport protocol (must maintain cool chain) the standard of storage (stored below temperature 8° months to collect the vaccine and they seldom follow Upazila Livestock Offices. Usually, it takes more than District Livestock offices, and then District Livestock Research Institute (LRI), Bangladesh, it distributed to After production of anthrax vaccine at Livestock (Huque et al., 2017; Saadullah, 2002). Bangladesh has and that continues over one year (Dipti et al., 2013; Hassan et al., 2015). The vaccine was proved to be this vaccine was conducted in a commercial farmed cattle named Lal Teer livestock Limited Mymensingh during the period from April 2013 to April 2014 (Hassan et al., 2015). The vaccine was proved to be effective in farmed goat and cattle, raising anti anthrax antibody and activates antibody production above the reference value at day 7 of post-vaccination and that continues over one year (Dipti et al., 2013; Roy et al., 2013; Hassan et al., 2015). Bangladesh has 24 million cattle, 26 million goats, 3.5 million sheep, and 1.5 million buffaloes (DLS, 2019) that has been playing a vital role in the socio-economic development but more than 80% are reared under the traditional farming system (Huque et al., 2017; Saadullah, 2002). After production of anthrax vaccine at Livestock Research Institute (LRI), Bangladesh, it distributed to District Livestock offices, and then District Livestock offices send it to the Upazila Livestock Offices. This is the vaccine distribution system of Department of Livestock (DLS), Bangladesh. Cattle farmers receive vaccines for their livestock immunization through Upazila Livestock Offices. Usually, it takes more than months to collect the vaccine and they seldom follow the standard of storage (stored below temperature 8° C) and transport protocol (must maintain cool chain) due to lack of facilities or other adversity like ice melt at vaccine carriers, use insufficient ice, traffic jams, uninterrupted power supply of refrigerator, etc. that sometime noticed by general peoples. So the farmers sometimes have suspicion on the quality of the vaccine. Most of the previous studies were conducted on crossbred cattle in organized dairy farms that maintained a minimum standard of feeding management under farming condition but at the rural community level, there has a wide range of diversity among bred, age, weight, feeding husbandry, and other management factors (Reuveny et al., 2001; Xu & Frucht, 2007; Dipti et al., 2013; Roy et al., 2013; Hassan et al., 2015). So, the present study was carried out to evaluate the humoral immune response of anthrax vaccine among the cattle of community households and to measure the impact of existing transportation and storage systems on the immunity status of anthrax immunized cattle using the Enzyme-Linked Immunosorbent Assay (ELISA) technique. 

Materials and Methods

Study area: The study was conducted in the Kamarkhandha Upazila of Sirajganj district, north-western Bangladesh, located in between 24°18' and 24°27' north latitudes and in between 89°35' and 89° 42' east longitudes (Figure 1) where “An integrated approach to establish an anthrax-free model area in Bangladesh” project was running from July 2018 to June 2020. This research was funded by the Ministry of Education (MoE), Government of the People’s Republic of Bangladesh, Project No: 2018/501/MoE.

Study group: For this study, a total of 60 cattle of different ages, breed, and sex from 60 farmers (1 from each farmer) were randomly selected from 3 separate parts of the studied Upazila. Most of them were between the ages of 1 to 3 years, bull and heifer calves that were not vaccinated before or not likely to become pregnant during the study period, were chosen. The selected cattle were divided into 3 groups each group comprised of 20 cattle. Of 3 groups of cattle, group 1(T1): vaccine collected from Livestock Research Institute, Group 2(T2): vaccine collected from District Livestock Office (Sirajganj), and Group 3 (T3): vaccine collected from Upazila Livestock Office (Kamarkhandha) were used.

Vaccination: The selected cattle were vaccinated subcutaneously two times, one just after the first blood sample collection and another were 10 months later of the first vaccination. The dose of each time was 1ml per cattle (each ml has approximately 1×107 attenuated live spores of Bacillus anthracis) and other measures were taken as per the manufacturer’s instruction. Commercial name of this vaccine was
Torka® vaccine. 5 ml blood sample from each animal was collected 6 times from all animals. After the collection of blood samples, serum was separated in sterilized epindrop tube and finally it was dispatched to the laboratory at the Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh maintaining a cold chain, according to standard protocol for ELISA. Serum separation and transportation methods were followed as Hassan et al., (2015) Serological analysis: The antibody level of serum samples was determined by the Enzyme-Linked Immunosorbent Assay (ELISA) to follow according to the manufacturer’s assay protocol [Nori® Bovine Anthrax Receptor 1 (ANTXR1) ELISA Kit, Cat. # GRC 112080, Genorise Scientific Inc, USA]. This kit was for the quantification of anthrax receptors in cattle. This was a quick ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for bovine of anthrax receptor was pre-coated onto a microplate. Standards and samples were pipetted into the wells and anthrax receptor was bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for bovine of anthrax receptor was added to the wells. Following a wash to remove any unbound antibody reagent, a detection reagent was added. After intensive wash, a substrate solution was added to the wells and color develops in proportion to the amount of anthrax receptor bound in the initial step. The color development was stopped and the intensity of the color was measured (Genorise, INC).

Data analysis: All the data of serum samples were recorded to excel spreadsheets and again categorized into 3 groups based on vaccine collection sites as T1 (Vaccine collected from Livestock Research Institute (LRI), Mohakhali, Dhaka, Bangladesh), T2 (Vaccine collected from District Livestock Office, Sirajganj), and T3 (Vaccine collected from Upazila Livestock office, Kamarkhandha, Sirajganj). Data were summarized into Microsoft Excel 7 (Microsoft Corporation, Redmond, WA, USA) spreadsheet and statistically analyzed using Epi-Info 3.5.3 (CDC, Atlanta, USA). Descriptive analysis was performed, and results expressed in frequencies and proportions. Categorical response variables were presented as proportions and their associations determined by chi-square tests and one-way analysis of variance (ANOVA).

Results

The ELISA values of IgG against anthrax vaccine were determined by Cutoff mode with wavelengths 405, 630nm. Before vaccination, the average ELISA value of serum sample was 0.18± 0.01, after vaccination with Sterne 34F-2 strain of *Bacillus anthracis* vaccine it was increased above the protective level (1.00) within one day.
month and continued up to the next vaccine schedule of next year. After the second dose (booster dose) of the vaccine, it further increased up to 2.34±0.08. Over time, the average ELISA value was statistically significant (p<.05) in 95% confidence intervals. The detailed findings are shown in Table 1-2 and Figure 2.

On the other hand, vaccines were categorized into three different groups based on their collection place. The ELISA value of the serum sample was divided into three groups as per the vaccine collection place. After analysis of the group-wise ELISA value, it was confirmed that the p-value was 0.98 which means there was no significant difference (p<.05) ELISA value among the three groups. The details findings are shown in Table 3 and Figure 3.

**Discussion**

In Bangladesh DLS under the Ministry of Fisheries and Livestock produces anthrax vaccine through LRI approximately 4.0 million doses of anthrax vaccine annually. However, the quantity of vaccines is not sufficient to immunize all susceptible animal species in Bangladesh. Due to a lack of resources like a shortage of manpower along with other facilities like a dedicated vaccine transportation system, proper cool chain are the constraints of a vaccination program (Sarker et al., 2013; Mondol and Yamag, 2014; Rahman et al., 2014). The main objective of this study was to evaluate the efficacy of the 34F-2 strain of the *Bacillus anthracis* vaccine at the farmers’ level. This strain has been used in different counties of the world to prevent anthrax disease in animals (Turnbull, 1998; Siamudala et al., 2006; Moazeni et al., 2007; Laxmi et al., 2016). The result of this study in the form of elevation of IgG anthrax vaccination in cattle was also confirmed by other researchers in Bangladesh (Dipti et al., 2013; Roy et al., 2013; Hassan et al., 2015). Moreover, individual variation of the immune response was noticed within the same species of different animals due to their biochemical and physiological differences (Glass et al., 1990; Koolhaas, 2008; Karim et al., 2010). The bovine T cell proliferation response was depending upon the major histocompatibility complex (MHC) class II (Petroff et al., 1997) for that reason the same dose of vaccine does not elicit the same amount of immune response that was also seen in this study. However, this finding is very insignificant therefore it could not affect our major finding. The successful development of protective immunity against anthrax vaccines in an animal requires a potent vaccine (Brey, 2005). In the 1930s, Sterne developed live, attenuated strains of *Bacillus anthracis*, which is still being used worldwide for immunization of domesticated animals against anthrax (Swartz, 2001). Further, vaccine response in household rearing cattle has been obtained in this study was corroborated by other researchers in Bangladesh (Dipti et al., 2013; Roy et al., 2013; Hassan et al., 2015). However, their study was conducted in commercially farmed animals.

| Time       | Before vac. | Month-1 | Month-4 | Month-7 | Month-10 | Month-13 | P- Value |
|------------|-------------|---------|---------|---------|----------|----------|----------|
| ELISA Value| 0.18 ± 0.01 | 2.16 ± 0.06 | 1.78 ± 0.06 | 1.61 ± 0.05 | 1.35 ± 0.04 | 2.34 ± 0.08 | 0.000    |

The data represent the mean value and ± standard error. Before vac. = Before vaccination.

![Figure 2. Antibody titer level of Sterne 34F-2 strain of *Bacillus anthracis* vaccine over time](image-url)
In this study, we found a minimum level of antibody titer elevation at the first blood sample (before vaccination), may due to got maternal antibodies from their mother (Turnbull et al., 1992; Lembo et al., 2011) or naturally got the infection through a low infective dose (WHO, 2008) since the study location is considered to be anthrax endemic area of Bangladesh (Islam et al., 2018).

**Conclusion**

Bangladesh Livestock Research Institute, Mohakhali, Dhaka produced Sterne 34F-2 strain of Bacillus anthracis vaccine is quite effective to produce sufficient immune responses. It also raised adequate immunity of the animal’s body against anthrax for a year and there was no impact on the immune response due to the existing transportation and storage system. Further study may compare this strain with other strains of the Bacillus anthracis vaccine at the community level is demanding as a future endeavor.

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Table 2. Groupwise ELISA value of serum samples according to collected Sterne 34F-2 strain of Bacillus anthracis vaccine from different place

| Time | Before vac. | Month-1 | Month-4 | Month-7 | Month-10 | Month-13 |
|------|------------|---------|---------|---------|----------|----------|
| ELISA values | T 1 | T 2 | T 3 | T 1 | T 2 | T 3 | T 1 | T 2 | T 3 | T 1 | T 2 | T 3 | T 1 | T 2 | T 3 |
| 0.16 | 0.11 | 0.02 | 2.14 | 2.9 | 2.12 | 1.53 | 1.2 | 1.82 | 1.47 | 1.2 | 2.52 | 1.11 | 1 | 1.59 | 2.34 | 1.57 | 1.84 |
| 0.17 | 0.12 | 0.18 | 2.75 | 1.5 | 2.36 | 1.5 | 1.73 | 1.86 | 1.37 | 1.96 | 1.36 | 1.01 | 1.63 | 1.56 | 2.56 | 1.85 | 2.36 |
| 0.15 | 0.13 | 0.5 | 2.56 | 1.42 | 2.46 | 1.52 | 1.53 | 1.76 | 1.4 | 1.64 | 1.06 | 1.2 | 1.43 | 2.46 | 2.66 | 1.66 | 3.18 |
| 0.23 | 0.17 | 0.37 | 2.36 | 1.37 | 2.34 | 1.49 | 1.67 | 1.23 | 1.37 | 1.97 | 1.19 | 1.26 | 1.57 | 2.04 | 2.56 | 1.84 | 2.81 |
| 0.23 | 0.16 | 0.38 | 3.15 | 2.38 | 2.57 | 1.95 | 1.58 | 1.97 | 1.78 | 1.78 | 1.37 | 1.68 | 1.48 | 1.57 | 3.12 | 1.74 | 2.35 |
| 0.3 | 0.15 | 0.12 | 1.79 | 1.54 | 2.56 | 1.56 | 1.74 | 1.39 | 1.45 | 1.94 | 1.36 | 1.22 | 1.64 | 1.06 | 1.92 | 1.89 | 2.13 |
| 0.11 | 0.16 | 0.21 | 1.45 | 2.53 | 2.34 | 1.45 | 1.63 | 1.54 | 1.33 | 1.73 | 1.74 | 1.22 | 1.53 | 1.04 | 1.68 | 1.79 | 2.11 |
| 0.12 | 0.26 | 0.16 | 1.68 | 1.46 | 1.23 | 1.5 | 1.86 | 1.73 | 1.42 | 1.26 | 1.23 | 1.22 | 1.76 | 1.23 | 1.58 | 2.12 | 2.19 |
| 0.1 | 0.4 | 0.2 | 1.84 | 2.1 | 2.45 | 1.68 | 1.78 | 2.55 | 1.5 | 1.5 | 1.65 | 1.3 | 1.13 | 1.18 | 1.72 | 2.7 | 2.16 |
| 0.15 | 0.5 | 0.1 | 1.65 | 2.23 | 3.56 | 1.6 | 1.43 | 2.13 | 1.3 | 1.63 | 2.09 | 1.2 | 1.19 | 1.07 | 1.75 | 2.93 | 2.87 |
| 0.11 | 0.12 | 0.11 | 1.84 | 2.11 | 3.1 | 1.45 | 2.31 | 1.2 | 1.32 | 2.51 | 1.3 | 1.15 | 1.56 | 1.16 | 2.11 | 2.43 | 2.7 |
| 0.08 | 0.11 | 0.1 | 3.14 | 1.28 | 1.23 | 2.5 | 1.88 | 1.33 | 2.1 | 1.48 | 1.03 | 1.5 | 1.68 | 1.33 | 3.33 | 1.99 | 2.13 |
| 0.11 | 0.11 | 0.1 | 3.12 | 2.39 | 1.35 | 2.41 | 1.89 | 1.15 | 2.11 | 1.39 | 1.05 | 1.5 | 1.69 | 1.45 | 3.12 | 2 | 2.25 |
| 0.21 | 0.15 | 0.13 | 3.01 | 2.37 | 1.25 | 2.88 | 2.27 | 1.65 | 2.34 | 1.27 | 2.05 | 1.96 | 1.07 | 0.95 | 3.22 | 2.42 | 1.78 |
| 0.12 | 0.19 | 0.13 | 2.96 | 2.21 | 1.24 | 2.15 | 2.31 | 1.94 | 1.98 | 1.41 | 1.64 | 1.44 | 1.11 | 1.14 | 3.11 | 2.5 | 2.51 |
| 0.15 | 0.23 | 0.15 | 2.45 | 2.34 | 1.23 | 2.03 | 1.54 | 2.13 | 1.98 | 1.74 | 2.03 | 1.4 | 1.34 | 1.33 | 2.49 | 2.77 | 2.58 |
| 0.14 | 0.22 | 0.1 | 3.12 | 2.89 | 2.68 | 2.45 | 1.99 | 2.38 | 1.45 | 1.39 | 2.08 | 1.21 | 1.09 | 1.58 | 3.5 | 3.21 | 2.92 |
| 0.21 | 0.41 | 0.21 | 1.45 | 3.1 | 1.98 | 1.23 | 1.45 | 2.78 | 1.11 | 1.3 | 2.58 | 1.01 | 1.3 | 1.29 | 1.85 | 3.61 | 2.11 |
| 0.22 | 0.03 | 0.15 | 1.24 | 2.92 | 2.23 | 1.22 | 1.23 | 1.43 | 1.11 | 1.12 | 1.63 | 1 | 1.02 | 1.53 | 1.22 | 3.05 | 2.58 |
| 0.14 | 0.03 | 0.23 | 1.04 | 1.57 | 2.12 | 1.12 | 2.17 | 2.22 | 1.21 | 1.77 | 2.32 | 1.1 | 1.31 | 1.32 | 1.26 | 2.2 | 1.59 |

**Mean** 0.16 0.19 0.18 2.24 2.13 2.12 1.76 1.76 1.81 1.56 1.60 1.66 1.28 1.38 1.39 2.36 2.31 2.36

**SD** 0.05 0.12 0.11 0.69 0.57 0.66 0.48 0.32 0.45 0.35 0.33 0.48 0.23 0.25 0.35 0.70 0.56 0.40

**SE** 0.14 0.36 0.79 0.47 0.33 0.45 0.38 0.29 0.33 0.29 0.30 0.30 0.22 0.25 0.28 0.46 0.45 0.29

**P-value** 0.67 0.82 0.92 0.79 0.79 0.43 0.96

**Remarks** NS NS NS NS NS NS NS

**Legends:** before Vac.: Before Vaccination; SD: Standard Deviation; SE: Standard Error; NS: Statistically not significant 1: Vaccine collected from Livestock Research Institute 2: Vaccine collected from District Livestock Office (Sirajganj), 3: Upazila Livestock Office (Kamarkand)
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Conflicts of Interest

The authors declare no conflict of interest.

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