JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

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

Shahjahan Ali Sarker, Ahosanul Haque Shahid, and K. H. M. Nazmul Hussain Nazir*

Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.
Sarker S. A. ORCID: 0000-0001-7480-3576 ; Shahid A. H.: ORCID: 0000-0002-0281-4644; K. H. M. Nazmul Hussain Nazir ORCID: 0000-0002-3893-754X

Research Article
Volume: 5, Issue: 1
April 2021
Pages: 32-38

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

Article History
Received: 04.02.2021
Accepted: 17.03.2021
Available online: 07.04.2021

DOI: https://doi.org/10.30704/http-www-jivs-net. 869532

To cite this article: Sarker S. A., Shahid A. H., and K. H. M. Nazmul Hussain Nazir 12021). Efficaciousness of Sterne 34F-2 strain of *Bacillus anthracis* vaccine in cattle for anthrax control program in Bangladesh. ***Journal of Istanbul Veterinary Sciences, 5*(1),** 31-38. **Abbreviated Title*: J. İstanbul vet. sci.***

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
E-mail: nazir@bau.edu.bd

Journal home page: www.jivs.net
http://dergipark.gov.tr/http-www-jivs-net

2004; Beyer & Turnbull, 2009; Thapa et al., 2014). Usually, herbivores (cattle, sheep, and goats) become infected when more than a specific quantity of spores enter the body with contaminated feedstuff in contaminated pastureland during grazing. Certain environmental factors such as drought, flooding rain, and soil tillage can all increase the risk of an anthrax outbreak in an area. On the other hand, weakly acidic loamy type soil (pH 6.38±0.15) with low organic matter like carbon and calcium level has positively influenced on anthrax incidence in animals. A human can become infected through direct or indirect contact with a sick animal only (Shafazand, et al., 1999; Chakraborty et al., 2012; Coffin et al., 2015; Islam et al., 2018). The preventive vaccination of livestock can manage the risk of anthrax infection. The anthrax vaccine, produced in Livestock Research Institute (LRI), Mohakhali, Bangladesh is a live spore vaccine. The master seed of this vaccine employed for this vaccine is Sterne 34F-2, which originated from Australia. It is effective immunologically to produce enough immunity to protect livestock against anthrax disease that was studied by Dipti et al., 2013 and Hasan et al., 2015. The morphological and immunological study was conducted on farmed goats at the Department of Pathology, Bangladesh Agricultural University during the period from 2012-2013 (Dipti et al., 2013), and another efficacy study of 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

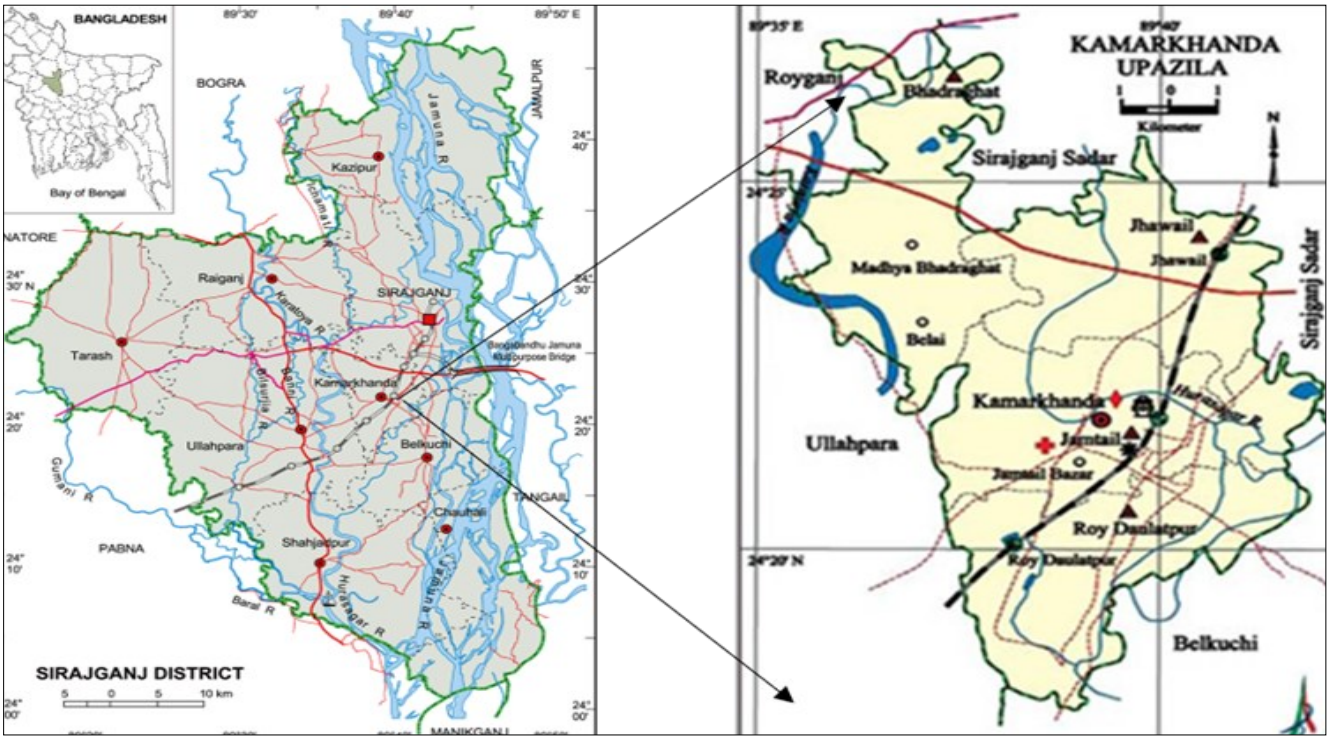

Figure 1. Location of the study area

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

Table 1. ELISA value of serum samples collected form the cattle vaccinated with F-34 strain of *Bacillus anthracis* vaccine

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

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 Yamage, 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; Siamudaala 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.

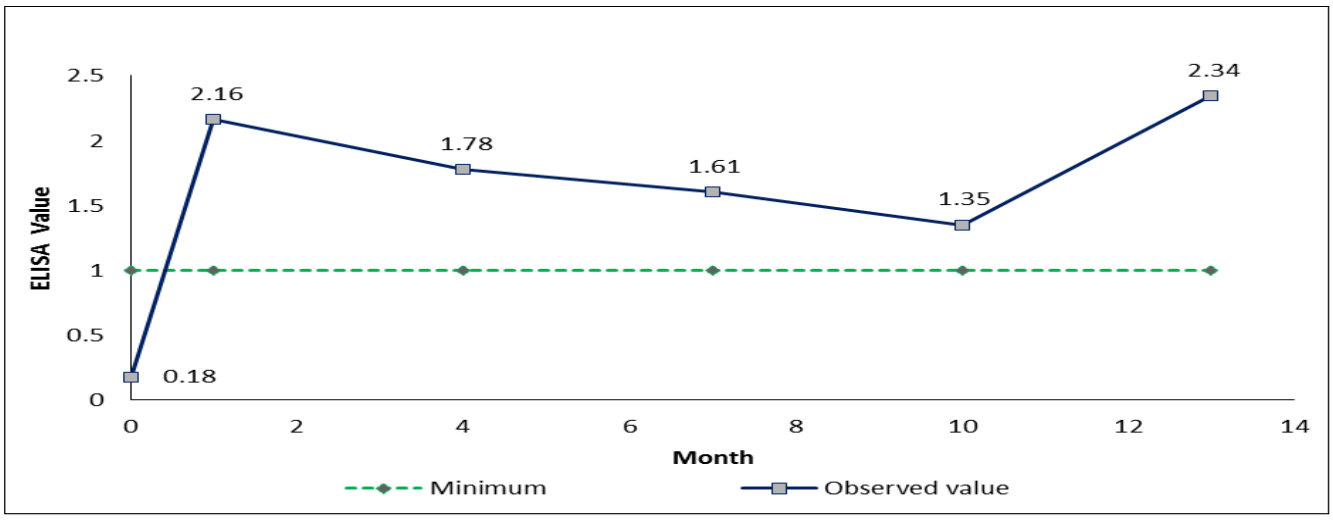

Figure 2. Antibody titer level of Sterne 34F-2 strain of *Bacillus anthracis* vaccine over time

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	T2	T3	$T1^1$	$T2^2$	$T3^3$	$T 1^1$	$T2^2$	$T3^3$	$T 1^1$	$T2^2$	$T3^3$	$T 1^1$	$T2^2$	$T3^3$	$T 1^1$	$T2^2$	$T3^3$
	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	1.11	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			0.43			0.96		
Remarks	NS			NS			NS			NS			NS			NS		

Legends: before Vac. : Befre Vaccination; SD:Stand Deviation; SE:stand Error; NS: Statistically not significant 1: Vaccine collected from Livestock Research Institute 2: Vaccine collected from District Livestock Office (Sirajganj), 3:Upazla Livestock Office (Kamarkand)

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.

Table 3. Comparative ELISA value of serum samples for the vaccine of three different places.

Vaccine collected form	Time of collection					P-value
	1st month	4th month	7th month	10th month	13th month	
Vaccine collected from Livestock Research Institute	2.24 ± 0.69	1.76 ± 0.48	1.56 ± 0.35	1.28 ± 0.23	2.36 ± 0.70	0,98
Vaccine collected from District Livestock Office (Sirajganj)	2.13 ± 0.57	1.76 ± 0.32	1.60 ± 0.33	1.38 ± 0.25	2.31 ± 0.56	
Upazila Livestock Office (Kamarkand)	2.12 ± 0.66	1.81 ± 0.45	1.66 ± 0.48	1.39 ± 0.35	2.36 ± 0.40	

Remarks: No significant difference exists between the three groups (p-value is larger than 0.05)

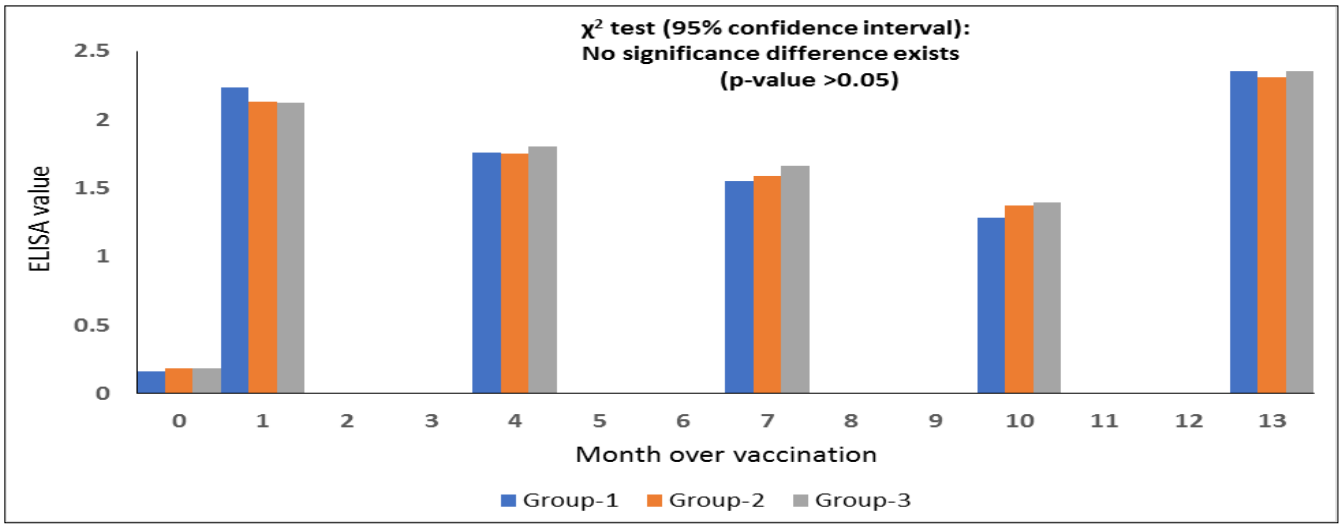

Figure 3. Compare of ELISA value between three groups (Group-1: Vaccine collected from Livestock Research Institute, Group-2: Vaccine collected from District Livestock Office and Group-3: Vaccine collected from Upazila Livestock office)

Acknowledgments

We are grateful to all people involved in this study for their valuable time, thought, opinion, positive criticism, advice, and suggestions. The authors extend to truthful acknowledgment to the District Livestock office, Upazila Livestock Office, Department of Livestock Services (DLS), Bangladesh, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh for their technical assistance. The authors acknowledge the Reviewers' contributions as they have significantly improved the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

Beyer, W. and Turnbull, P. (2009). Anthrax in animals. *Molecular Aspects of Medicine, 30*(6), 481-489.

Brey, R. N. (2005). Molecular basis for improved anthrax vaccines. *Advanced drug delivery reviews, 57*(9), 1266-1292.

Chakraborty, A., Khan, S. U., Hasnat, M. A., Parveen, S., Islam, M. S., Mikolon, A., ... & Hossain, M. J. (2012). Anthrax outbreaks in Bangladesh, 2009–2010. *The American journal of tropical medicine and hygiene, 86*(4), 703-710.

Coffin, J. L., Monje, F., Asiimwe-Karimu, G., Amuguni, H. J., & Odoch, T. (2015). A One Health, participatory epidemiology assessment of anthrax (*Bacillus anthracis*) management in Western Uganda. *Social Science & Medicine,* 129, 44-50.

Dipti, M. A. D. H. A. B. I., Rashid, M. M., Ferdoush, M. J., Roy, P., Khan, M. A. H. N. A., & Hossain, M. M. (2013). Morphological and immunological characterization of anthrax vaccine in cattle. *Bangladesh Journal of Veterinary Medicine, 11*(1), 43-49.

DLS. (2019). Livestock Economy at a Glance. Department of Livestock Services, Ministry of Fisheries and Livestock, Government of the People's Republic of Bangladesh. Available online:http://dls.portal.gov.bd/sites/default/files/files/dls.portal.gov.bd/page/ee5f4621_fa3a_40ac_8bd9_898fb8ee4700/Livestock Economy at a glance %282017-2018%29.pdf

Genorise Scientific, INC, PO Box 70, Glen Mills, PA 19342 USA Web adress: www.genorise.com.

Glass, E. J., Oliver, R. A., & Spooner, R. L. (1990). Variation in T cell responses to ovalbumin in cattle: evidence for Ir gene control. *Animal genetics, 21*(1), 15-28.

Hassan, J., Rahman, M. B., Chowdhury, S. M. Z. H., Rabidas, Sand immunological characterization of anthrax spore vaccine in goat. Bangladesh *Journal of Veterinary Medicine, 11*(2), 151-157..

Hugh-Jones, M., & Blackburn, J. (2009). The ecology of *Bacillus anthracis*. *Molecular Aspects of Medicine, 30(*6), 356-367.

Huque, K. S., Khanam, J. S., Amanullah, S. M., Huda, N., Bashar, M. K., Vellinga, T., & Hicks, K. (2017). Study on existing livestock manure management practices in Bangladesh. *Current Journal of Applied Science and Technology,* 1-10.

Islam, S. S., Chakma, S., Akhter, A. T., Ibrahim, N., Talukder, F., & Chowdhuary, G. A. (2018). Investigation of animal anthrax outbreaks in the human–animal interface at risky districts of Bangladesh during 2016–2017. *Journal of advanced veterinary and animal research, 5*(4), 397.

Karim, Z., Huque, K. S., Hussain, G., Ali, Z., & Hossain, M. (2010). Growth and development potential of livestock and fisheries in Bangladesh. Government of the People's Republic of Bangladesh.

Koolhaas, J. M. (2008). Coping style and immunity in animals: making sense of individual variation. *Brain, Behavior, and Immunity, 22*(5), 662-667.

Laxmi, U., Chaturvedi, V., Gupta, P., Sunita, S., Sumithra, T. (2016). Morphological and Physio-Chemical Characteristics of an Isolate of *Bacillus anthracis* (IVRI Strain). *Journal of Veterinary and Public Health,* 14, 13-18.

Lembo, T., Hampson, K., Auty, H., Beesley, C. A., Bessell, P., Packer, C., ... & Cleaveland, S. (2011). Serologic surveillance of anthrax in the Serengeti ecosystem, Tanzania, 1996–2009. *Emerging infectious diseases, 17*(3), 387.

Moazeni, J. G. R., Jabari, A., Vahedi, D. F. (2007). Determination of anthrax foci through isolation of *Bacillus anthracis* form soil samples of different regions of Iran. *Archives of Razi Institute,62*(1),23-30.

Mondal, S. P., & Yamage, M. (2014). A retrospective study on the epidemiology of anthrax, foot and mouth disease, haemorrhagic septicaemia, peste des petits ruminants and rabies in Bangladesh, 2010-2012. *PloS one, 9*(8), e104435.

Petroff, M. G., Mark Coggeshall, K., Jones, L. S., & Pate, J. L. (1997). Bovine luteal cells elicit major histocompatibility complex class II-dependent T-cell proliferation. *Biology of reproduction, 57*(4), 887-893.

Rahman, S., Begum, I. A., & Alam, M. J. (2014). Livestock in Bangladesh: distribution, growth, performance and potential. *Livestock Research and Rural Development,* 26, 233-238.

Reuveny, S., White, M. D., Adar, Y. Y., Kafri, Y., Altboum, Z., Gozes, Y., Kobiler, D., Shafferman, A., & Velan, B. (2001). Search for correlates of protective immunity conferred by anthrax vaccine. *Infection and Immunity, 69*(5), 2888-2893.

Roy, P. R., Rashid, M. M., Ferdoush, M. J., Dipti, M., Chowdury, M. G. A., Mostofa, M. G.,& Hossain, M. M. (2013). Biochemical and immunological characterization of anthrax spore vaccine in goat. *Bangladesh Journal of Veterinary Medicine, 11*(2), 151-157.

Saadullah, M. (2002). Smallholder dairy production and marketing in Bangladesh. Smallholder dairy production and marketing-Opportunities and constraints. Nairobi, Kenya: NDDB (National Dairy Development Board) and ILRI (International Livestock Research Institute), 7-21.

Saile, E., & Koehler, T. M. (2006). *Bacillus anthracis* multiplication, persistence, and genetic exchange in the rhizosphere of grass plants. *Applied and Environmental microbiology, 72*(5), 3168-3174.

Sarkar, S., Hossain, M. M., & Amin, M. R. (2013). Socio-economic status of buffalo farmers and the management practices of buffaloes in selected areas of Bagerhat District of Bangladesh. *Bangladesh Journal of Animal Science,* 42 (2), 158-164.

Shafazand, S., Doyle, R., Ruoss, S., Weinacker, A., & Raffin, T. A. (1999). Inhalational anthrax: epidemiology, diagnosis, and management. *Chest, 116*(5), 1369-1376.

Shiferaw, G. (2004). Anthrax in Wabessa village in the Dessie Zuria district of Ethiopia. *Revue Scientifique et Technique-Office International des Epizooties, 23*(3), 951-956.

Siamudaala, V. M., Bwalya, J. M., Munang'andu, H. M., Sinyangwe, P. G., Banda, F., Mweene, A. S., ... & Kida, H. (2006). Ecology and epidemiology of anthrax in cattle and humans in Zambia. *Japanese Journal of Veterinary Research, 54*(1), 15-23.

Silvestri, E. E., Perkins, S. D., Feldhake, D., Nichols, T., & Schaefer, F. W. (2015). Recent literature review of soil processing methods for recovery of *Bacillus anthracis* spores. *Annals of microbiology, 65*(3), 1215-1226.

Swartz, M. N. (2001). Recognition and management of anthrax—an update. *New England Journal of Medicine, 345* (22), 1621-1626.

Thapa, N. K., Tenzin, K. W., Tshering Dorji, M., Dorjee, J., Marston, C. K., & Hoffmaster, A. R. (2014). Investigation and control of anthrax outbreak at the human–animal interface, Bhutan, 2010. *Emerging Infectious Diseases, 20* (9), 1524.

Turnbull, P. C. B., Doganay, M. E. H. M. E. T., Lindeque, P. M., Aygen, B. İ. L. G. E. H. A. N., & McLaughlin, J. (1992). Serology and anthrax in humans, livestock and Etosha National Park wildlife. *Epidemiology and Infection, 108*(2), 299-313.

Turnbull, P. C. (1998). Organization, World.Health. Guidelines for the surveillance and control of anthrax in humans and animals, WHO, Department of Communicable Diseases Surveillance.

Xu, L., & Frucht, D. M. (2007). *Bacillus anthracis*: a multi-faceted role for anthrax lethal toxin in thwarting host immune defenses. *International Journal of Biochemistry and Cell Biology, 39*(1), 20-24.

WHO. (2008). Anthrax in humans and animals. 4th Edition. Retrived from: https://apps.who.int/iris/bitstream/handle/10665/97503/9789241547536_eng.pdf