CHAPTER-I
INTRODUCTION

Anthrax is an acute infectious zoonotic disease caused by the spore-forming, aerobic, gram-positive, non-motile bacterium *Bacillus anthracis*. Among the three clinical forms of anthrax in humans, more than 95% of naturally occurring infections are cutaneous anthrax. Gastrointestinal anthrax is usually caused by the consumption of insufficiently cooked contaminated meat, and it is relatively uncommon. Inhalation anthrax is rare in naturally occurring infections and is related to processing and handling hides and wool in enclosed factory spaces, where aerosolized anthrax spores may be inhaled (Chakraborty *et al.*, 2012)

In Bangladesh, the unprecedented epidemic trend of anthrax outbreaks affecting both bovine animals and humans were recorded in the districts of Pabna and Sirajganj in the monsoon months of two consecutive years, 2009 and 2010. During the years 1980–1984 in the Pabna milk shed locations, 62 animal illnesses were reported, with 69% of the animals dying. (Ahmed *et al.*, 2010). In another report, 333 animal instances were discovered during 1989 to 1996. There were 29 human anthrax outbreaks in 2009 and 2010, with 706 cases in 12 areas (Ahmed *et al.*, 2010).

During August 2009–October 2010, a collaborative team of epidemiologists, physicians, veterinarians, and anthropologists from the Institute of Epidemiology, Disease Control and Research (IEDCR), the Department of Livestock Services of the Government of Bangladesh, and the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b) conducted 14 anthrax outbreak investigations. The objectives of these outbreak investigations were to identify the etiology, modes of transmission, the social, behavioral, and cultural factors that contributed to these outbreaks, and suggest control and prevention measures (Chakraborty *et al.*, 2012).

Now many European countries, North America, and Australia have controlled anthrax, and the disease is now absent or only sporadic in those countries. In Africa, Zambia was identified as a model country of the anthrax control program. Regular vaccinations, increased public awareness, and proper quarantine were considered a major strategy to control anthrax in Zambia (Sarker *et al.*, 2020).

Anthrax is linked to contact with sick livestock, especially during slaughtering. When interacting with an animal or animal product, the risk may be increased if the hands have cuts on them (Woods *et al.*, 2004).

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=48) from Sirajganj, Bangladesh throughout the year 2012. Approximately 400-gm of surface soil from a maximum depth of one-feet was collected according to the procedure, whereas the results of the endospore-positive samples fell into the following ranges: 6.31-28.37%, 5.17-7.22, 484.35-1372.35 ppm, and 0.15-2.35%. (Ahsan *et al.*, 2013). Anthrax occurrence was linked to an increase in soil pH that was more alkaline too (Kracalik *et al.*, 2017).

The majority of the outbreaks occurred during the monsoon season when heavy rainfall occurred. Most of the cattle owners mentioned that they did not have dry land away from the household premises to bury the carcasses during the monsoon season, so they discarded the carcasses either in the flood waters or in the river (Islam *et al.*, 2013).

Independent risk factors for anthrax in cattle in the nation included recent slaughter of sick animals on the farm or a nearby farm (odds ratio (OR) 12.2, 95% CI: 1.6-93.4, P = 0.016), history of heavy rains occurring in the two weeks prior to an outbreak (OR 13.1, 95% CI 1.2-147.1, P = 0.037), and disposal of dead animals into nearby water bodies (OR 11.9, 95% CI 1.0-145.3, P =0.052). Also, "feeding animals with water hyacinth (*Eichhornia crassipes*)" was identified as an independent risk factor (Rume *et al.*, 2020).

Despite the effectiveness of cattle vaccines in preventing anthrax, underreporting, logistical challenges, and a lack of funding made it challenging to carry out immunization campaigns. In previous studies, advanced statistical models showed that vaccination, the use of antibiotics during an outbreak, and the duration of vaccine delivery were significantly ($p<0.05$) predicted occurrence of anthrax outbreak (Mongoh *et al.*, 2008).

Though anthrax outbreak is repetitively happening in Bangladesh in both human and animals, we found very little published documents on vaccine production and distribution practices in home and abroad. Again, factors affecting anthrax outbreaks was also not evaluated extensively hence contributing to the knowledge gap in tropical country like Bangladesh. Anthrax has serious public health significance. So, we need to determine the production and distribution channel of anthrax vaccine from Livestock Research Institute (LRI) to the Upazilla level of Bangladesh. The common practice of vaccine shipment in Bangladesh is mostly one or two messenger come from the district level with an authorization letter of required number of doses of vaccine needed to LRI and receive the vaccines with ice packs. After that, it might become challenging to maintain the cool chain while travelling by the local transport. After that at different levels to the way of vaccine recipient end, the deviation of cool chain might affect the effectiveness of anthrax vaccine in food animals of Bangladesh. Again, so many

superstitions against receiving animal anthrax vaccine by farmers are in place. By finding out the factors affecting anthrax vaccine intake in Meherpur district of Bangladesh, we may draw a possible explanation of repetitive anthrax outbreak in animals and human in the area.

OBJECTIVES:

The overall aim of the study was to evaluate the production and distribution channel and practices of anthrax vaccine to identify the factors might affect the intake of vaccine in livestock population of Bangladesh. Hence the specific objectives were-

- To understand the production and distribution practices of anthrax vaccine in Bangladesh.
- To identify the factors affecting the intake of anthrax vaccine in livestock population of Meherpur.

CHAPTER-II
REVIEW OF LITERATURE

2.1 Anthrax and its etiology

Anthrax is an acute infectious zoonotic disease caused by the spore-forming, aerobic, gram-positive, non-motile bacterium *Bacillus anthracis*. Among the three clinical forms of anthrax in humans, more than 95% of naturally occurring infections are cutaneous anthrax. Gastrointestinal anthrax is usually caused by the consumption of insufficiently cooked contaminated meat, and it is relatively uncommon. Inhalation anthrax is rare in naturally occurring infections and is related to processing and handling hides and wool in enclosed factory spaces, where aerosolized anthrax spores may be inhaled (Chakraborty *et al.*, 2012)

2.2 Transmission pathway

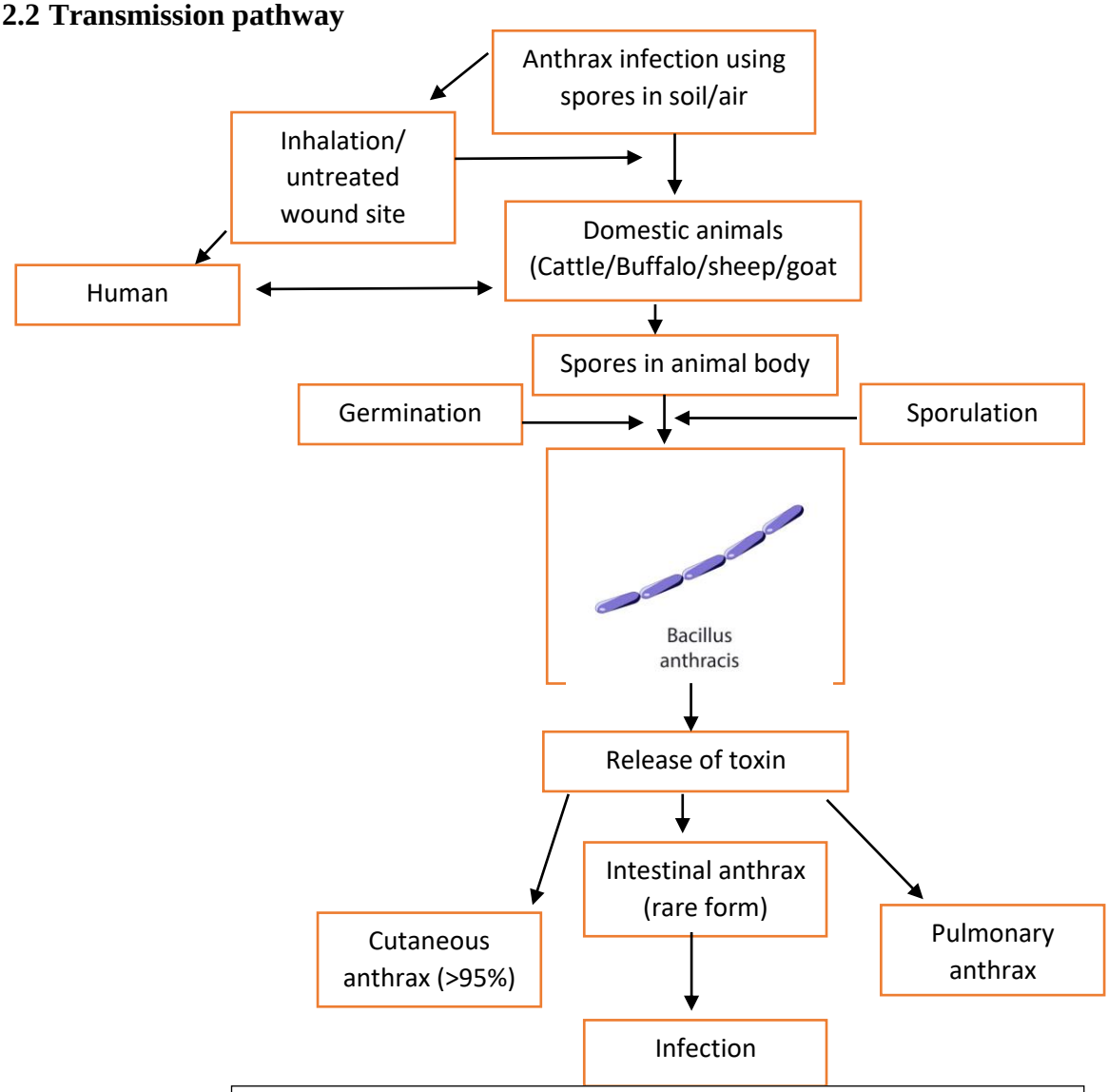

Fig 1: Transmission pathway of anthrax in human and animals

2.3 Prevalence of anthrax

Anthrax spore can stay alive in soils for years without infecting animals i.e. stopping organism-spore-organism cycle. In May and November, the anthrax outbreak is high in trend considering 2400 suspected human cases with at least 36 outbreaks since 2009 in Bangladesh specially in and around Sirajganj Districts. (National Bulletin of Public Health, Volume 1, Issue 4, March-2019)

There were 11 anthrax occurrences in Bangladesh in 2011 in six different districts. In order to identify and classify Bacillus anthracis (B. anthracis) strains, various samples were gathered from May to September in the six regions where anthrax had occurred. In 46.6% of the samples analyzed, particularly in soils, but also in bone samples, water, and animal feed, anthrax was found. This study makes the assumption that contaminated feed and water can have a significant impact on the epidemiology of anthrax, and that soil is not the sole factor contributing to the disease's transmission in Bangladesh. There is discussion of potential explanations for these epidemiological connections.(Galante *et al.*, 2021)

In France, so distant, as it were two expansive episodes of *Bacillus anthracis* caused by infusion have been portrayed, influencing basically Northern European nations in two isolated waves crossing 2009–2010 caused 1668 animal cases and 122 human cases and in 2012–2013, causing 70 cases and 26 fatalities. (Thouret *et al.*, 2020)

2.3.1 Human anthrax

Anthrax outbreaks involving 140 animal cases and **273 human cases** in 14 anthrax-affected villages were investigated by the International Centre for Diarrheal Disease Research, Bangladesh (icddrb) and the Institute of Epidemiology, Disease Control and Research between August 2009 and October 2010. The goals of that inquiry were to investigate the circumstances behind these outbreaks, including livestock rearing practices, how people handled sick and dead animals, and the anthrax immunization program. (Islam *et al.*, 2013)

The unprecedented epidemic trend of anthrax outbreaks affecting both bovine animals and humans was recorded in the Districts of Pabna and Sirajganj in monsoon months of two consecutive years, 2009 and 2010. During the years 1980–1984 in the Pabna milk shed locations, 62 animal illnesses were reported, with 69% of the animals dying. In another report, 333 animal instances were discovered.1989 to 1996, coming from Bangladesh. There were ***29 human anthrax*** outbreaks in 2009 and 2010, with 706 infections in 12 areas. (Ahmed *et al.*, 2010).

Five of the 22 people who contracted anthrax in 2001 died as a result of their illness. The illness that killed them was inhalation anthrax, which is the most lethal form of the disea se. A total of 4

people werediscovered to have anthrax infections, and an additional 10,000 people were thought to be at risk of anthrax exposure. (Against *et al.*, 2011)

2.3.2 Animal anthrax

In Bangladesh, cases on smallholdings of ***anthrax-affected cattle*** were enrolled for a matched case-control study from October 2010 to December 2014. The cases were first made public by the media and/or in surveillance reports from the relevant national authorities. There were 43 case smallholdings enrolled in all. With respect to herd size and animal raising, a control was chosen for each case from a location that was remote (within 3–10 km) but still fell under the same sub-district of the case farm. A prototype questionnaire was used to collect data, which was then analyzed using matched-pair analysis and multi-variable conditional logistic regression. Of the 43 smallholdings, 41 were found in three adjacent districts: Pabna, Sirajganj, and Tangail, which may have formed a spatial cluster and been referred to as a "hot zone" for anthrax in Bangladesh. Independent risk factors for anthrax in cattle in the nation included recent slaughter of sick animals on the farm or a nearby farm (odds ratio (OR) 12.2, 95% confidence interval (CI) 1.6-93.4, P = 0.016), history of heavy rains occurring in the two weeks prior to an outbreak (OR 13.1, 95% CI 1.2-147.1, P = 0.037), and disposal of dead animals into nearby water bodies (OR 11.9, 95% CI 1.0-145.3, P =0.052). (Galante *et al.*, 2021)

Table-1: Reported Animal and Human anthrax cases in Bangladesh (Ahmed *et al.*, 2010)

Year	Animal Case	Human Case
1980	6	0
1981	7	0
1982	16	17
1983	21	10
1984	12	0
1989-96	333	0
1997	0	19
Oct 2009-June 2010	55	99
Aug 2010-Oct 2010	140	607
Total	590	752

2.4. Risk factors of animal and human anthrax

The majority of the outbreaks occurred ***during the monsoon season*** when heavy rainfall occurred. Most of the cattle owners mentioned that they did not have dry land away from the household premises to bury the carcasses during the monsoon season, so they discarded the carcasses either in the flood waters or in the river. (Islam *et al.*, 2013)

A collaborative team of epidemiologists, physicians, veterinarians, and anthropologists from the Institute of Epidemiology, Disease Control and Research (IEDCR), the Department of Livestock Services of the Government of Bangladesh, and the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b) conducted the outbreak investigations. The objectives of these outbreak investigations were to identify the etiology, ***modes of transmission, the social, behavioral, and cultural factors that contributed to these outbreaks***, and suggest control and prevention measures (Chakraborty *et al.*, 2012).

A cross sectional study was conducted to create awareness against anthrax for early detection and management in Rajshahi Medical College in 2011. The primary bases for the suspicion of anthrax were cutaneous signs of the classic non-tender ulcer with black eschar, with or without edema, and a history of dressing, washing, or butchering cattle, goats, or their meat. By displaying big gram-positive rods that often-resembled *B. anthracis* under a microscope, the diagnosis was made. The average age of the cases was

21.4 years (with a range of 3 to 46 years), with 7 (46.7%) men and 8 (53.3%) women involved. Most of the cases came from unaware and economically insolvent families. Butchering (20%), coming into touch with raw meat (46.7%), and handling live animals (33.3%) were among the exposure types. The frequencies of malignant pustule were 11 (73.3%), 2 (13.3%), 1 (6.7%), and 1 in the upper extremity, both extremities, face, and trunk, respectively (Siddiqui et al., 2012).

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-feet was collected according to the procedure (Ahsan *et al.*, 2013).

A soil's ability to support spore survival depends in great part on its calcium content and pH level, both of which are high in anthrax-prone areas. It is hypothesized that the spore exosporium likely plays a significant role by limiting dispersal and so raising the likelihood that a grazing animal may receive a deadly amount. Hot, ***dry weather*** during "Anthrax Seasons" ***stresses*** animals and lowers their ***natural resistance to infection***, making little amounts of spores infectious. ***Haemophagic flies serve as space-multipliers*** whereas ***necropsied flies serve as case-multipliers***; the latter are supported by environmental conditions that are crucial in determining whether epidemics take place. The species' sensitivity to the toxins determines whether the host dies. ***Scavengers***' primary job is to open the carcass, leak fluids and help bacilli spread and start sporulation as a result. In terms of landscape ecology, the distribution of viable spores is influenced by factors like elevation, mean NDVI, yearly NDVI amplitude, soil moisture content, and soil pH. (Hugh-Jones and Blackburn, 2009)

Independent risk factors for anthrax in cattle in the nation included recent slaughter of sick animals on the farm or a nearby farm (odds ratio (OR) 12.2, 95% confidence interval (CI) 1.6-93.4, P = 0.016), history of heavy rains occurring in the two weeks prior to an outbreak (OR 13.1, 95% CI 1.2-147.1, P = 0.037), and disposal of dead animals into nearby water bodies (OR 11.9, 95% CI 1.0-145.3, P =0.052). Study identified "Feeding animals with water hyacinth (*Eichhornia crassipes*)" an independent risk factor (Rume et al., 2020)

2.5 Prevention and control

Now many European countries, ***North America, and Australia have controlled anthrax***, and the disease is now absent or only sporadic in those countries. In Africa, ***Zambia was***

identified as a model country of the anthrax control program. ***Regular vaccinations, increased public awareness, and proper quarantine*** were considered a major strategy to control anthrax worldwide. (Sarker *et al.*, 2020).

The majority of the research concurred that insufficient vaccination rates were a significa nt

factor that contributed to anthrax infection, and the research advised that consistent and e ffective

immunization could lower the chance of acquiring anthrax. ('WHO_CDS_VPH_93.117 very important document the south african control measure.pdf', 1992)

Mainly being a disease affecting animals, the management of anthrax preventing the spread in cattle, sheep, and goats, is crucial for maintaining the health of b oth animals and humans. The *B. anthracis* strain 34F2, which was created by Max Sterne in 1937, is commonly included in many animal anthrax vaccines. (Adone *et al.*, 2016)

According LRI, the F24 strain of *B. anthracis* which is of Australian origin as master seed protects goat from anthrax by administering 0.5ml subcutaneously once a year. (Roy *et al.*, 2014)

A mathematical modeling was described by the researchers of Ghana in 2020 to explore and control the infectious disease where the numerical simulation showed the control measures like vaccination, education, disinfection and treatment can play important role in preventing and controlling diseases. (Joshua *et al.*, 2020)

2.5.1 Human vaccination

According to a study on human anthrax vaccination for the laboratory personnel, the immunity of combined (inhalation & cutaneous) vaccine efficacy on two doses completion showed 93% (David L. Sewell, 2003).

Concerns about the current human vaccination strategy have led to improvements in the current vaccination strategies, along with the quest for additional immunogens, creation of novel adjuvants, novel delivery mechanisms, and agents for a safer and more effective immunization program. An easy-to-use or efficient anthrax vaccine that could hasten the onset of a protective immune response is desirable, particularly for post-exposure prophylaxis. Below is a description of experimental anthrax vaccines (Table 1) that are various stages of research or safety and immunogenicity evaluation.

Table 2: Selected experimental human anthrax vaccines (Kaur, Singh and Bhatnagar, 2013)			
Vaccine name	*Immunogen/Composition*	*Status*	*R&D or manufacturer*
Epicutaneous anthrax vaccine	PA and germination-associated anthrax antigens	Under development	Vaxin Inc.
Intranasal anthrax vaccine	PA and germination-associated anthrax antigens	Pre-clinical testing	Vaxin Inc.
rPA102	PA	Phase II clinical trials	Vax Gen
rPA vaccine (Thraxine™)	PA	Phase II clinical trials	Avecia Biotechnology Pharm Athene
Novel anthrax vaccine	Psoralen-killed metabolically-active vaccine	Preclinical research and development	Cerus
Dry anthrax vaccine	PA	Phase I clinical trials	Iomai
AV7909	AVA adjuvanted with CpG oligonucleotides	Phase I clinical trials	Emergent Bio Solutions
Improved targeted anthrax vaccine	Human monoclonal antibody specific for mannose receptors as a delivery vehicle for anthrax PA	Under development	Medarex
AVA: Anthrax vaccine adsorbed PA: Protective antigen; rPA: Recombinant PA			

In order to immunize A/J mice against a model of inhalation anthrax, we employed irradiated anthrax spores. Irradiated spore-vaccine preparations from bacteria with or without the gene expressing PA provided defense against the toxin-producing Sterne strain challenge. For the vaccine to be effective, the mucosal adjuvant, CT, was needed. T-cell depletion and serum transfer experiments performed on immunized mice at the time of an infectious challenge showed that CD4 T cells were essential during the effector phase of protection. IL-17, but not IFN-, IL-5, or IL-10, was generated by spore-specific CD4 T cells after mucosal vaccination with CT and irradiation anthrax spores. (Datta *et al.*, 2010)

In previous studies, advanced statistical models showed that vaccination, the use of antibiotics during an outbreak, and the duration of vaccine delivery were significantly ($p<0.05$) predicted occurrence of anthrax (Mongoh *et al.*, 2008).

2.5.2 Animal vaccination

A group of researchers showed that it is possible to combine immunization campaigns for nomadic pastoralists and their cattle in Chad between 2000 and 2005. The expenditures were lower overall since veterinarians and doctors shared transportation logistics and equipment. The adaptation to and high value of joint delivery of human and animal health care pastoralists in remote areas. More women and children were vaccinated per day during joint vaccination rounds than during vaccinations of just individuals without vaccinating their livestock (130 vs. 100, p0.001), which resulted in the first time that 10% of nomadic children (>1-11 months of age) in intervention zones were fully immunized annually. Public health and veterinary services, particularly at the district level, are made more effective by making the most use of their limited human and logistical resources. (Schelling et al., 2007)

According to Annual Performance Agreement (APA) 2021-2022 of Department of Livestock Services (DLS), had been produced about 32 crore doses of vaccine for the domestic and pet animals where a good amount of anthrax vaccine was anthrax vaccines for animals. Within the capacity and demand of the field offices, the Livestock Research Institute (LRI) Mohakhali and Cumilla has been producing anthrax vaccine with other vaccines to prevent diseases in livestock of Bangladesh. Again, a good number of doses are also imported by the non-government sector every year. (APA report 2021-2022, DLS)

2.5.3 Treatment

Since many years ago, penicillin has been the preferred treatment for anthrax, and only very rarely has penicillin failed. Isolates that are discovered naturally include resistance. In vitro, *B. anthracis* is also susceptible to the majority of other widely used antibiotics, including clindamycin, imipenem, rifampin, vancomycin, cefazolin, tetracyclines, chloramphenicol, macrolides, aminoglycosides, and other first-generation cephalosporins. It is resistant to cefuroxime, aztreonam, trimethoprim, and sulfamethoxazole, as well as extended-spectrum cephalosporins such cefotaxime and ceftazidime. In contrast to the later and weaker EF- and PA-specific IgG responses, the majority of the toxin-specific antibody responses noticed after infection in human were directed against LF, with immunoglobulin G (IgG) found as early as 4 days after the onset of symptoms. The majority of the toxin-specific antibodies produced by those inoculated with the US anthrax vaccine absorbed and the UK anthrax vaccine precipitated approved anthrax vaccines were directed against PA, in contrast to the case with infection. They found that human antibodies specific for LF were, like anti-PA

antibodies, able to block toxin activity, raising the idea that they might be protective. Hence they came to the conclusion that an antibody response to LF may serve as a more accurate diagnostic indicator of anthrax than PA. (General and Swartz, 2001)

2.5.4 Challenges of preventing anthrax

Despite the effectiveness of ***cattle vaccines*** in preventing anthrax, ***underreporting, logistical challenges, and a lack of funding*** made it challenging to carry out immunization campaigns. Anthrax occurrence was linked to an increase in soil pH that was more alkaline too (Kracalik *et al.*, 2017).

A study was carried out in Burkina Faso to estimate the ***cost of vaccination***. The study found that the cost of labor is the largest contributor to inputs, accounting for 65% of all costs. Compared to the private sector, which is expected to bear 26% of the cost, fixed expenses are greater in the public sector by up to 46%. This study assists veterinary services in their options for better resource allocation in Burkina Faso and the Sahel to undertake PPR and ***other small ruminant disease*** management programs. (Guy Sidwatta Ilboudo *et al.*, 2022)

CHAPTER III

MATERIALS AND METHODS

3.1 Study area

Meherpur district is an Anthrax endemic region of the country where both human and animal anthrax outbreaks reported periodically. Therefore, this study was conducted in 3 upazillas (Meherpur sadar, Gangni and Mujibnagar) of Meherpur district.

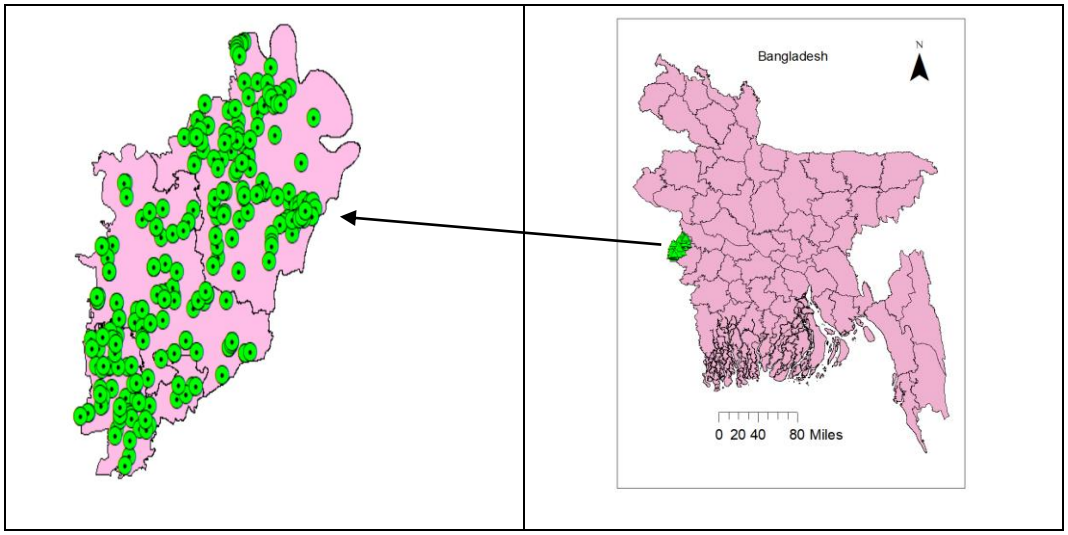

Fig 2: Farm level map of cattle (Meherpur)

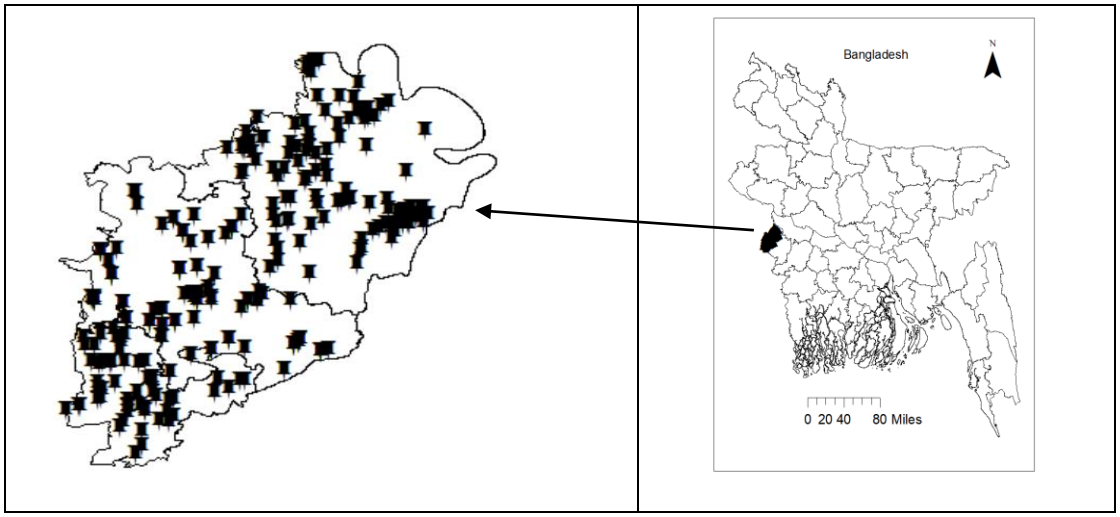

Fig 3: Farm level map of goat (Meherpur)

3.2 Feasibility Test

It was done through a 5-days visit to different upazillas of Meherpur district. On that visit, a series of activities were done eg. collection of livestock population data, anthrax data recorded in livestock from the Veterinary Hospital, visiting the anthrax prone areas, collection of records of upazilla wise vaccine coverage against population, Key

Informant's Interview (KII) etc. For LRI, the demand, production and distribution (criteria based) records were collected for the last 5 years.

All the information gathered were cleaned and stored for identifying the loop holes like limitations in questionnaire, time count for every interview etc. and later addressed to meet the study criteria before starting final data collection. Full preparation was taken for the series of activities and noted for future.

3.3 Questionnaire preparation

The questionnaire was prepared by addressing the objectives of the study. A thorough literature review was conducted to identify potential factors to be investigated. The questionnaire was pre-tested on a small group of the population and amended necessarily. There were four different types of semi-structured questionnaire used for the data collection. Type-I was for the Livestock Research Institute (LRI) head of Mohakhali, Dhaka and Cumilla. Type-II was for DD, DLO and ULO; Type-III was designed for SALO, CEAL, LSP, and Vaccinator; Type-IV was constructed for the farmers. Data were collected from the interviewees through face-to-face interview in a descriptive manner. General information and vaccine production & distribution related information were collected through Type-I questionnaire.

3.4 Sampling of study unit

3.4.1 ***Target population***- All ruminants (Cattle-11,47,789; Buffalo-20,783; Goat-226,298; Sheep-10,880) of Meherpur district.

3.4.2 Sampling frame- A list of cattle and goat farms from three different Upazilla Livestock Office of Meherpur were collected.

3.4.3 Sampling strategy- We selected the cattle farms comprising at least 3 cattle and goat farms comprising at least 5 goats from the sample frame. After meeting the inclusion and exclusion criteria, the farms were selected using stratified random sampling (by using random calculator) irrespective of age, breed and sex of livestock in the study area. We stratified the farms into two strata as cattle and goat farms and then employed simple random sampling to select individual farms.

3.4.4 Sample size - The formula used for sample size calculation was as follows-

$$\text{Sample size, } n = N * \frac{\frac{Z^2 * p * (1-p)}{e^2}}{[N - 1 + \frac{Z^2 * p * (1-p)}{e^2}]}$$

Where ‘n’= Sample size

N= Population size

Critical Value (95% level of significance) ‘Z’ = 1.96

Margin of error ‘e’= 0.05 and Sample proportion ‘p’=0.5

N.B. If the prevalence is not known, then sample proportion can be assumed as 50%. (‘Sample size Calculator.pdf’, no date)

3.4.4.1. Mujibnagar-

Cattle farm sample size ‘n’ = $69*\{1.96^2*0.5(1-0.5)/0.0.05\}/ [69-1+ \{1.96^2*0.5(1-0.5)/0.05\}] = 59$

Goat farm sample size ‘n’ = $75*\{1.96^2*0.5(1-0.5)/0.05\}/ [75-1+ \{1.96^2*0.5(1-0.5)/0.05\}] = 63$

From a total of 69 cattle farms having at least 3 cattle and a total of 75 goat farms having at least 5 goats**, 59 cattle farms** and **63 goat farms** were selected randomly as the study group.

3.4.4.2. Gangni-

Cattle sample size ‘n’ = $172*\{1.96^2*0.5(1-0.5)/0.05\}/ [172-1+ \{1.96^2*0.5(1-0.5)/0.05\}] = 120$

Goat sample size ‘n’ = $240*\{1.96^2*0.5(1-0.5)/0.05\}/ [240-1+ \{1.96^2*0.5(1-0.5)/0.05\}] = 148$

A total of 172 cattle farms having at least 3 cattle and a total of 240 goat farms having at least 5 goats, **120 cattle** and **148 goat farms** were selected randomly as study group using the sample size calculator.

3.4.4.3. Meherpur Sadar-

Cattle sample size ‘n’ = $200*\{1.96^2*0.5(1-0.5)/0.05\}/ [200-1+ \{1.96^2*0.5(1-0.5)/0.05\}] = 132$

Goat sample size ‘n’ = $115*\{1.96^2*0.5(1-0.5)/0.05\}/ [115-1+ \{1.96^2*0.5(1-0.5)/0.05\}] = 89$

A total of 200 cattle farms having at least 3 cattle and a total of 115 goat farms having at least 5 goats, we selected **132 cattle and 89 goat farms**, respectively as study group using the sample size calculator.

Hence a grand total of **311 cattle and 300 goat farms** were selected for interview.

3.4. Data management

There were qualitative and quantitative data generated from four different types of questionnaires. Both types of data were stored in Microsoft excel worksheet and refined

for further processing. Qualitative data were described in detail in the relevant sections. Quantitative data were organized, cleaned and categorized when necessary, using standard procedure. The linearity of the quantitative variables was evaluated by categorizing them into four categories using the quartiles as cut-off values. Logistic regression analysis was conducted on the categorized variables, and parameter estimates were observed for an increasing or decreasing trend. In the case of linear increase or decrease in the parameter estimates, linearity in the quantitative variable was assumed and used without modification. In the case of nonlinearity, a quartile was used to categorize it. For instance, age of the owner has been divided into four categories on the basis of quartiles. However, educational status of farm owner has been classified into three, primary occupation into two, and experience of farming into three and four for cattle and goat farmers, respectively according to the research interest.

3.5. Statistical data analysis

Data from a total of 311 cattle and 300 goat farms were used in the analysis. After data cleaning and management, distribution of a total of 17 variables were shown in tables. To conduct the significance tests, variables were chosen by considering the plausibility of having an effect on the outcome variable. The effect of different potential explanatory variables on the binary outcome—vaccine intake/not—was evaluated using χ^2 test. P-values < 0.05 were considered as significant throughout the analysis. STATA-IC 17.0 (Stata Corp, College Station, TX, USA) and Microsoft Excel were used for statistical analyses and visualization.

CHAPTER-IV

RESULTS

4.1 Production of vaccine

Anthrax vaccine is produced in Livestock Research Institute (LRI), Mohakhali, Dhaka and Cumilla by the government. In 2020-2021 and 2021-2022, the two organizations jointly had an Annual Performance Agreement (APA) target of producing 80 lakh doses (LRI-60 lakh doses and Cumilla 20 lakh doses) of anthrax vaccine for each fiscal year. Again, in the fiscal year 2022-2023 it has been decreased to 72 lakh doses (LRI-54 lakh doses and Cumilla-18 lakh doses).

Institutions	Fiscal Year 2020-2021 Production	Fiscal Year 2021-2022 Production	Fiscal Year 2022-2023 Production
LRI	60 lakh doses		
Cumilla	20 lakh doses		
LRI		60 lakh doses	
Cumilla		20 lakh doses	
LRI			54 lakh doses
Cumilla			18 lakh doses

4.2 Distribution of vaccine

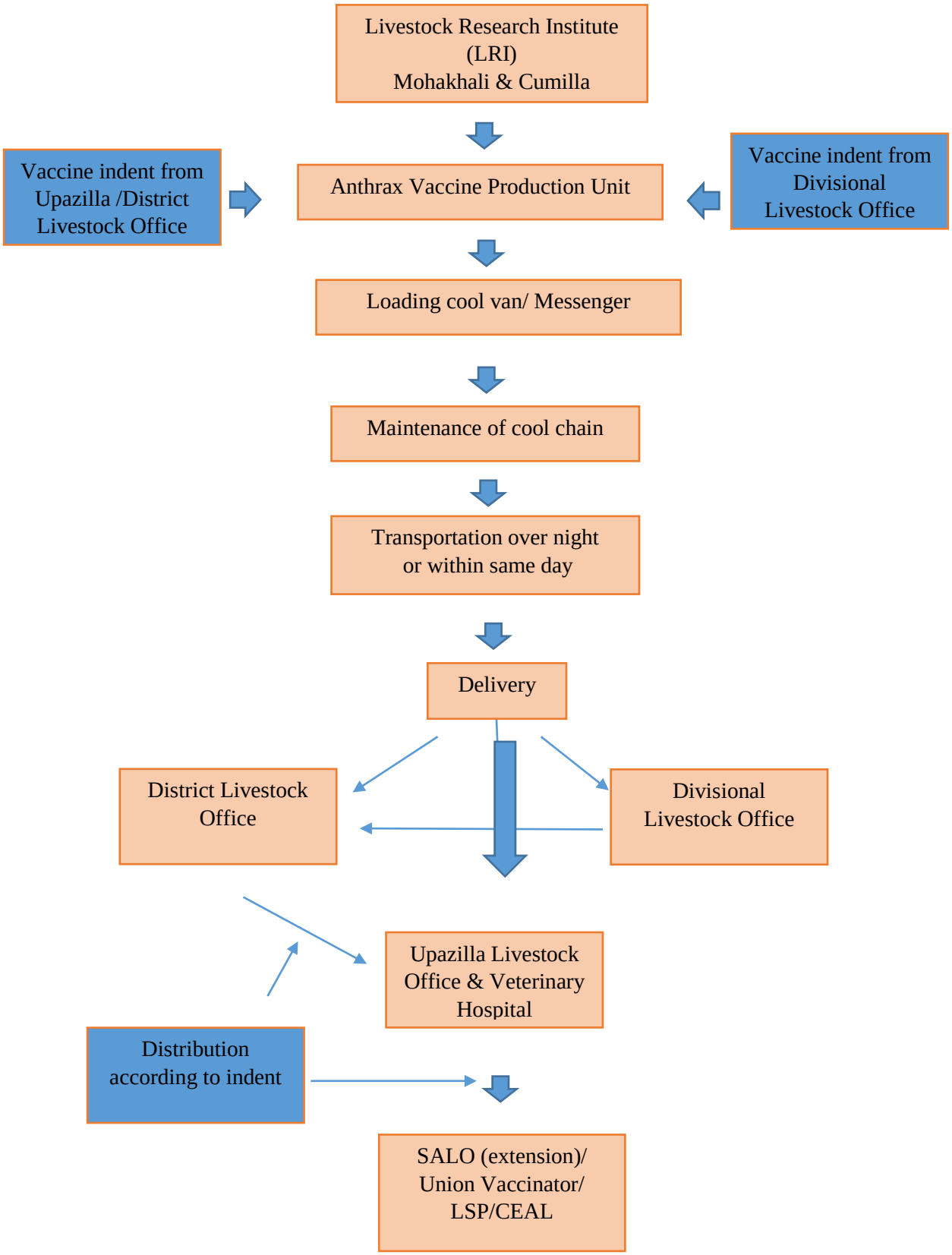

Fig 4: Diagram of existing vaccine distribution

4.3 Descriptive statistics of the study population

We selected a total of 17 parameters for both cattle and goat farms namely gender of farm owner, age, Educational status, primary occupation, experience of the farming, source of vaccine, use of anthrax vaccine, unusual event occurred after vaccine, animal death in last 6 months, allowance of animal in grazing pasture etc. which may describe the merit of this study.

4.3.1. For cattle farm

Table 3: Descriptive statistics of the cattle farmers

Sl. No.	Category	Content	Frequency	Percentages
1.	Age	23 to 34	83	26.69
		35 to 43	74	23.79
		44 to 56	78	25.08
		57 to 78	76	24.44
2.	Gender	Male	117	37.62
		Female	194	62.38
3.	Occupation	Farming	93	29.90
		Others	218	70.096
4.	Education level	Illiterate	180	57.87
		Up to primary	86	27.65
		HSC and above	33	10.61
5.	Purpose of farming	Milk	113	36.33
		Meat	149	47.91
		Mixed	49	15.76
6.	Farming experience	1 to 7 years	87	27.97
		8 to 12 years	127	40.84
		13 to 32 years	97	31.19
7.	Know about anthrax disease	Yes	56	18.006
		No	255	81.99
8.	Heard about anthrax from	Neighbour	2	0.64
		Local Market	1	0.32
		Upazilla	14	4.50

Sl. No.	Category	Content	Frequency	Percentages
		Veterinary Hospital		
		Quack	36	11.57
		Didn't hear	258	82.96
9.	Seen anthrax spread	Yes	9	2.89
		No	302	97.10
10.	Know about anthrax vaccine	Yes	8	2.57
		No	303	97.42
11.	Source of information about anthrax vaccine	Upazilla Veterinary Hospital	2	0.64
		Quack	6	1.92
		Don't know	303	97.43
12.	Vaccine to own livestock	Yes	107	34.41
		No	204	65.59
13.	Source of anthrax vaccine	Upazilla Veterinary Hospital	31	9.97
		Quack	79	25.40
		Not Given	201	64.43
14.	Unusual event after vaccination	Yes	33	10.61
		No	77	24.75
		Not given	201	64.63
15.	Animal death in last 6 months	Yes	1	0.32
		No	310	99.68
16.	Animal allowed in green pasture	Yes	25	8.03
		No	286	91.96
17.	Season of vaccination	Monsoon or pre/post monsoon	8	2.57
		Summer or	25	8.04

Sl. No.	Category	Content	Frequency	Percentages
		pre/post summer		
		Winter or pre/post winter	62	19.94
		Spring or pre/post spring	15	4.82
		Not given	201	64.63

** NB. (High school and above= High school, HSC, Vocational, Madrasha, Tertiary and others, Age category was done on the basis of minimum and maximum frequency i.e. 23-78 yrs)

Table 4: Descriptive statistics of the goat farmers

Sl. No.	Category	Content	Frequency	Percentages
1.	Age	19 to 33	75	25
		34 to 36	78	26
		37 to 54	77	25.67
		55 to 78	70	23.33
2.	Gender	Male	95	31.67
		Female	105	35
3.	Occupation	Farming	60	20
		Others	240	80
4.	Education level	Illiterate	170	56.67
		Up to primary	90	30
		HSC and above	40	13.33
5.	Purpose of farming	Milk	6	2
		Meat	283	94.33
		Mixed	11	3.66
6.	Farming experience	1 to 4 years	86	28.67
		5 to 8 years	66	22
		9 to 13 years	89	29.67
		14 to 32 years	59	19.67

Sl. No.	Category	Content	Frequency	Percentages
7.	Know about anthrax disease	Yes	29	9.67
		No	271	90.33
8.	Heard about anthrax from	Neighbour	17	5.67
		Local Market	2	0.67
		Upazilla Veterinary Hospital	6	2
		Quack	4	1.33
		Didn't hear	271	90.33
9.	Seen anthrax spread	Yes	299	99.66
		No	1	0.34
10.	Know about anthrax vaccine	Yes	27	9
		No	273	91
11.	Source of information about anthrax vaccine	Upazilla Veterinary Hospital	26	8.67
		Quack	64	21.33
		Don't know	210	70
12.	Vaccine to own livestock	Yes	90	30
		No	210	70
13.	Source of anthrax vaccine	Upazilla Veterinary Hospital	26	8.67
		Quack	64	21.33
		Not Given	210	70
14.	Unusual event after vaccination	Yes	28	9.33
		No	62	20.66
		Not given	210	70
15.	Animal death in last 6 months	Yes	5	1.63
		No	295	98.33
16.	Animal allowed in	Yes	21	7

Sl. No.	Category	Content	Frequency	Percentages
	green pasture	No	279	93
17.	Season of vaccination	Monsoon or pre/post monsoon	8	2.67
		Summer or pre/post summer	20	6.67
		Winter or pre/post winter	26	8.67
		Spring or pre/post spring	15	5
		Not given	211	70.33

** NB. (High school and above= High school, HSC, Vocational, Madrasha, Tertiary and others, Age category was done on the basis of minimum and maximum frequency i.e. 19-78 yrs)

4.4 Factors affecting the low vaccine intake

4.4.1. For cattle population

Table 5: Association of different factors on vaccine intake in cattle population

Outcome variable: Vaccine against anthrax to own livestock				
Categories	**Level**	**No (%)**	**Yes (%)**	**P-Value**
Gender of farm owner	Male	79 (67.52)	38 (32.48)	0.57
	Female	125 (64.43)	69 (35.57)	
Age of farm owner	23 to 34	56 (67.47)	27 (32.53)	0.34
	35 to 43	49 (66.22)	25 (33.78)	
	44 to 56	45 (57.69)	33 (42.31)	
	57 to 78	54 (71.05)	22 (28.95)	
Educational status of farm owner	Illiterate	125 (69.44)	55 (30.56)	0.002*
	Up to Primary	60 (69.77)	26 (30.23)	
	HSC and above	19 (42.22)	26 (57.78)	
Primary occupation	Farming	60 (64.72)	33 (35.48)	
	Others	144 (66.06)	74 (33.94)	
Experience of	1 to 7 years	63 (72.41)	24 (27.59)	0.10

farming	8 to 12 years	85 (66.93)	42 (33.07)	
	13 to 32 years	56 (57.73)	41 (42.27)	
Know about anthrax	No	168 (65.88)	87 (34.12)	0.82
	Yes	36 (64.29)	20 (35.71)	
Knowledge on anthrax sign in livestock	No	191 (66.09)	98 (33.91)	0.50
	Yes	13 (59.09)	9 (40.91)	

4.4.2. For goat population

Table 6: Association of different factors on vaccine intake in goat population				
		Vaccine against anthrax to own livestock		
Categories	**Level**	**No (%)**	**Yes (%)**	**P-Value**
Gender of farm owner	Male	74 (77.89)	21 (22.11)	0.04*
	Female	136 (66.34)	69 (33.66)	
Age of farm owner	19 to 33	45 (60.00)	30 (40.00)	0.03*
	34 to 36	57 (73.08)	21 (26.92)	
	37 to 54	62 (80.52)	15 (19.48)	
	55 to 78	46 (65.71)	24 (34.29)	
Educational status of farm owner	Illiterate	122 (71.76)	48 (28.24)	0.33
	Up to Primary	64 (71.11)	26 (28.89)	
	HSC and above	24 (60.00)	16 (40.00)	
Primary occupation	Farming	41 (68.33)	19 (31.69)	0.75
	Others	169 (70.42)	71 (29.58)	
Experience of farming	1 to 4 years	62 (72.09)	24 (27.91)	0.69
	5 to 8 years	49 (72.24)	17 (25.76)	
	9 to 13 years	60 (67.42)	29 (32.58)	
	14 to 32 years	39 (66.10)	20 (33.90)	
Know about anthrax	No	193 (71.22)	78 (28.78)	0.15
	Yes	17 (58.62)	12 (41.38)	
Knowledge on anthrax sign in livestock	No	197 (70.36)	83 (29.64)	0.61
	Yes	13 (65.00)	7 (35.00)	

4.5 Descriptive comparison of different variables according to farm type

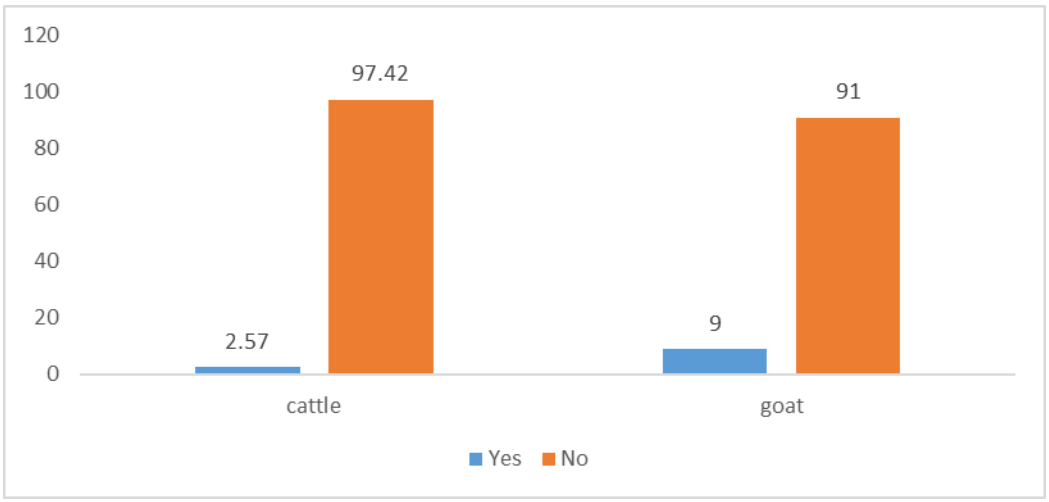

Fig 5: Farmer's knowledge about anthrax in cattle and goat

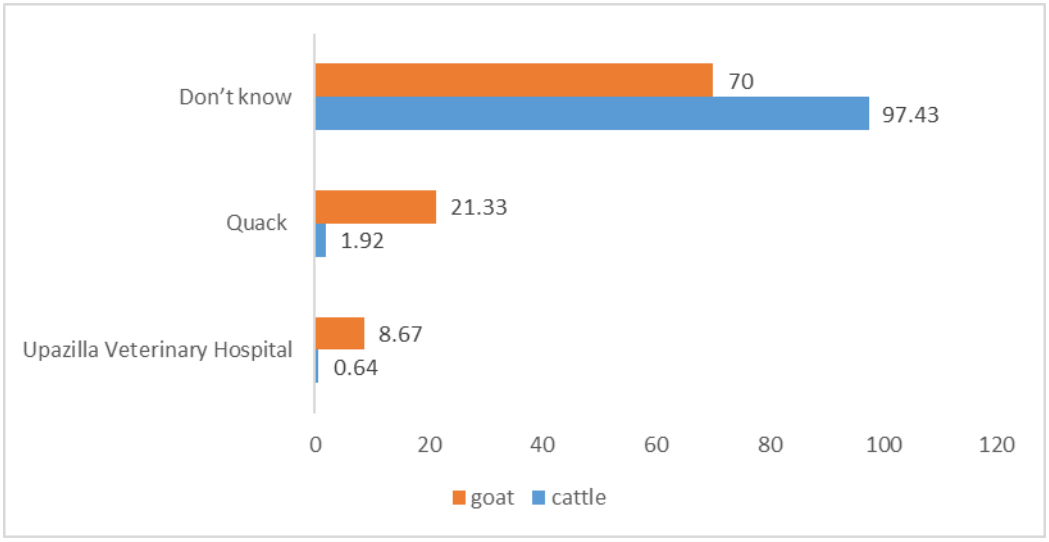

Fig 6: Source of information about anthrax vaccine in cattle and goat

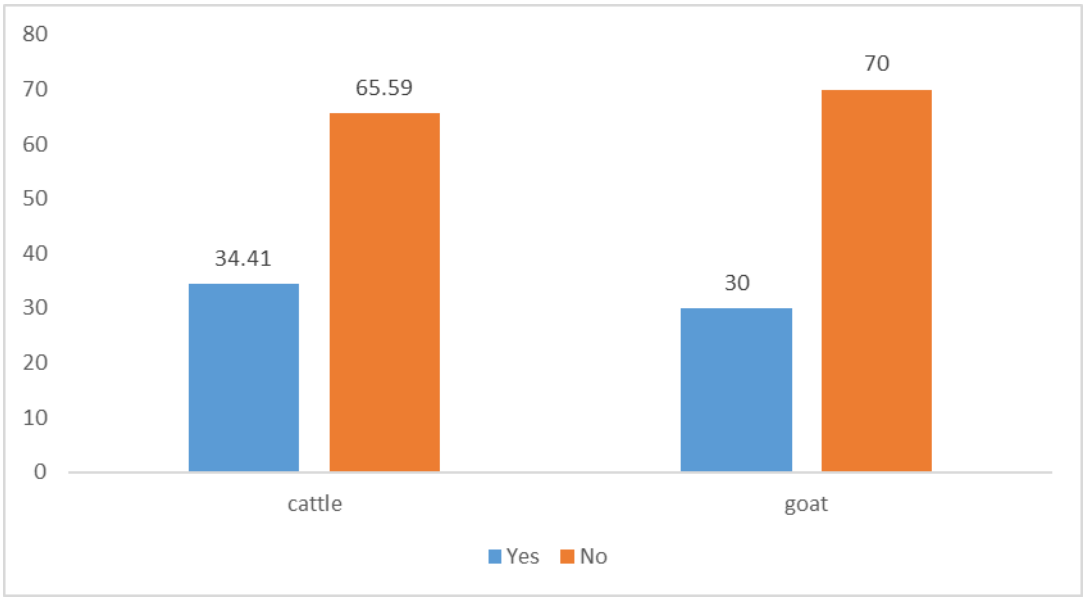

Fig 7: Vaccine own livestock by the owner of cattle and goat

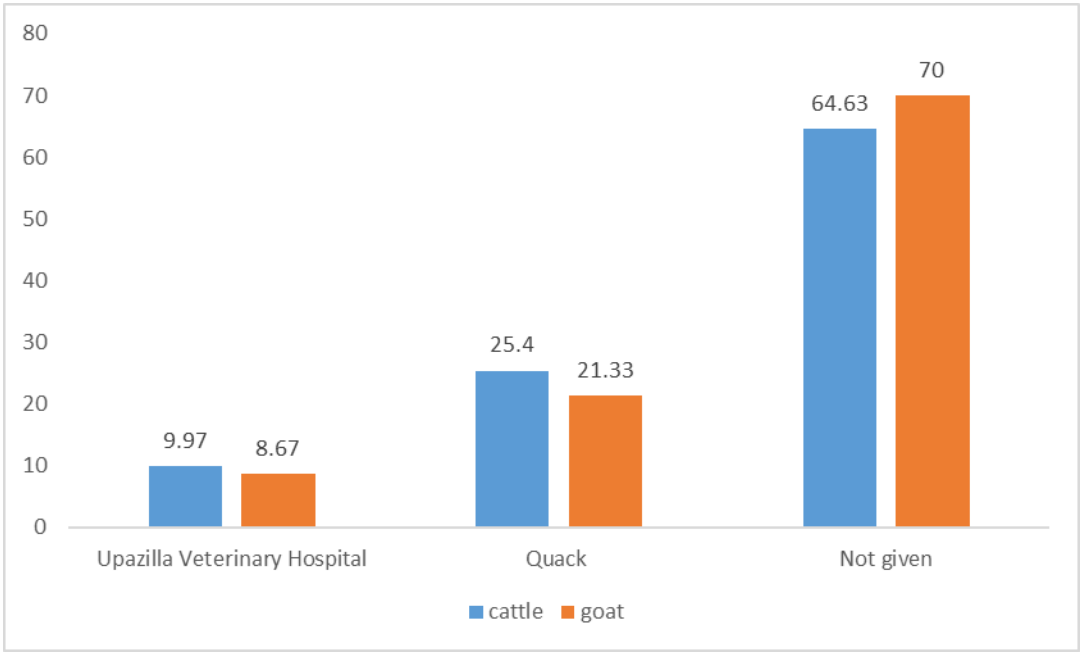

Fig 8: Farmer's source of anthrax vaccine for cattle and goat

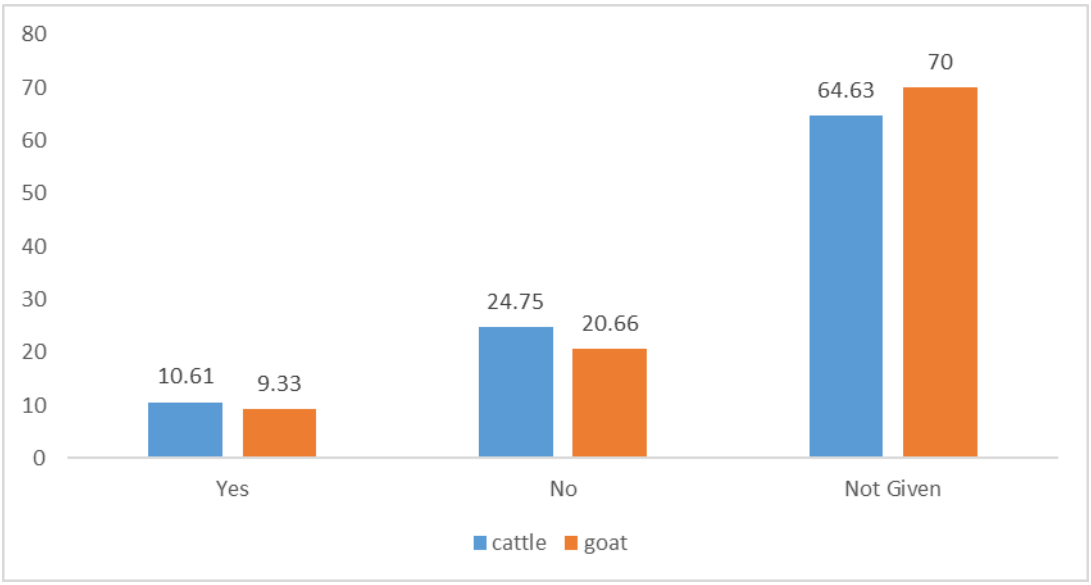

Fig 9: Unusual event occurred after anthrax vaccine in cattle and goat

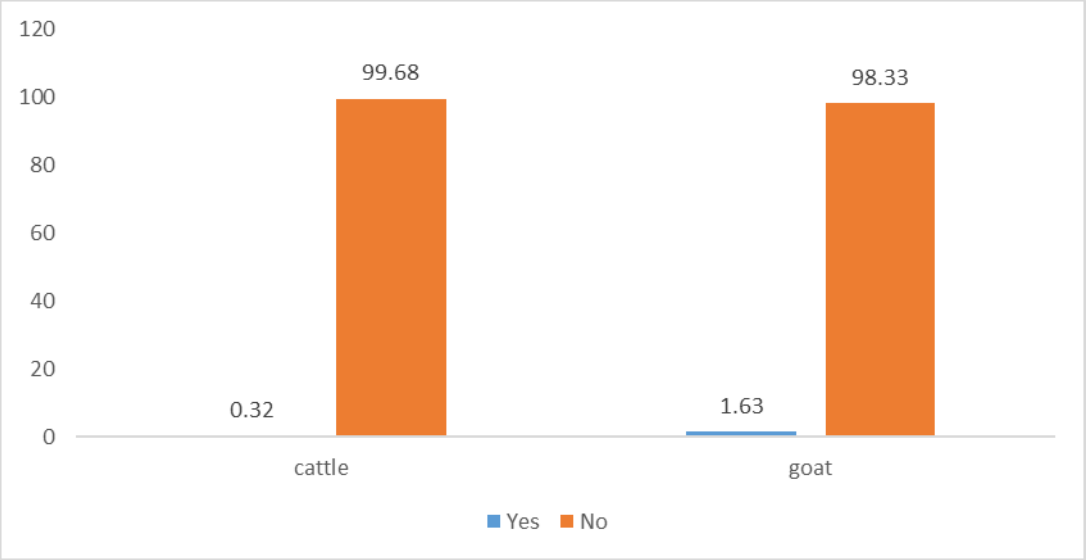

Fig 10: Animal death in last 6 months by anthrax in cattle and goat

CHAPTER-V

DISCUSSION

Both the manpower and machinery capacity of production has been decreased from the fiscal year 2021-2022 to 2022-2023. But the amount produced against the target fixed during the opening of fiscal year is dependent on previous year's production, demand and distribution trend. Sometimes master seed availability, curtail of budget and some other factors like unavailability of glassware, manpower shortage etc. may hamper the amount of production of anthrax vaccine.

The distribution channel of anthrax vaccine in Bangladesh was investigated through KII. By evaluating the existing distribution channel of anthrax vaccine from production unit to the field level, we can track the way vaccines distributed to all over Bangladesh. One is working as a subunit of Central Vaccine Production Unit, LRI, Mohakhali, Dhaka and another one is a subunit under the LRI, Cumilla. The vaccines are usually distributed against the indent submitted by the District Livestock Offices who inturn receives indent from the respective Upazillas. Sometimes Divisional Livestock Offices may give indent according to districts demand. Again, in special cases like outbreak situation and different project implementations, Upazilla Livestock Office and Veterinary Hospital may give indent with the permission of proper authority to those anthrax vaccine production units. All these demands are made upon the Annual Performance Agreement (APA) and for some cases production capacity of the respective anthrax vaccine production units. Once vaccines are produced, those are surely distributed to the field. Because government gets revenue from the vaccine sales. Here anthrax prone areas (Meherpur, Sirajganj, Pabna, Gaibandha etc) does not get the privileges of having more vaccines. Moreover, the total population of cattle, buffalo, sheep and goats of respective geographic areas are not considered while giving indent.

Two ways of transport systems are involved in the whole process. One is central cool van of LRI, Mohakhali distributes vaccines in listed districts on regular basis. Another way is messenger from respective districts come with indent paper and receive the vaccines in their personal cool box transporting it by their own arrangement.

In both cases, they distribute vaccines to the respective Upazillas. The Upazilla distribute it by Sub Assistant Livestock Officer (Extension)/Community Extension Agent for Livestock (CEAL)/Livestock Service Provider (LSP)/ Union Vaccinators. Government distributes the vaccines at a concession price and Upazilla office pay back through government treasury challan (figure 3).

Table 05 shows the results of $\chi 2$ (chi-square) test used to evaluate the relationships between explanatory variables and the dependent variable - vaccine against anthrax to own livestock. The null hypothesis (Ho) was there is no relationship. Statistically significant relationship between gender and age of the farmers with the intake of anthrax vaccine to their cattle was not evident from our analysis.

A strong relationship between vaccine against own livestock and educational status of the farm owner of cattle was revealed. We found that highest percentage (57.78%) of farmers with a degree of HSC and higher level provided vaccine to their herds. Thirty percent of the cattle farmers with no schooling and having up to a primary level education provided vaccine to their own livestock.

It was observed that if the farmers were more experienced (13 to 32 years of experience) a greater percentage of them provided vaccine to their herds; the test was not statistically significant though. The chi square test between 'vaccine against own livestock' to 'primary occupation of the owner' was not statistically significant and thus less intake of anthrax vaccine is not dependent on primary occupation of the owner.

Results revealed that there is no relationship between knowing about anthrax by the farmers with the intake of anthrax vaccine to their cattle. It may be due to unawareness and lack of communication with the Upazilla Livestock Office.

The Pearson's chi square test on 'vaccine against anthrax to own livestock vs knowledge on anthrax sign in livestock had an insignificant p-value ($P>0.05$) which indicates there is no correlation between the variables. It means knowledge may not depend on vaccination status of own livestock against anthrax. In all three Upazilla Livestock Office there are few projects, under which they give free anthrax vaccines to the livestock. But the farmers usually don't know much about it.

Unlike cattle farm owners, we observed a statistically significant relationship between gender and age of the farm owner with the outcome variable – vaccine against own livestock. It was observed that more female owners vaccinated their goats compared to male. Similarly, it was seen that 19 to 33 years' age group people provided more anthrax vaccine to their goats.

Unlike cattle farm owners, educational status of farm owner had no significant relationship with the outcome variable, though it was found that 40% of the farmers having a degree of HSC and above provided vaccine to their goats compared to 28% of the farmers having an educational level from illiterate up to primary.

The distribution of the variables ‘primary occupation’ and ‘experience of farming’ showed no variation according to levels of the outcome variable and the p-value of the chi square test was not significant. More of the farmers (41%) with knowledge about anthrax vaccinated their goats compared to the farmers had no knowledge (28%).

Figure 4 shows that a smaller number of cattle farmers know about anthrax than the goat farmers. Moreover, the cattle farmers get minimum information about anthrax from Upazilla Veterinary Hospital than that of goat farmers (Figure 5).

Though the cattle farmers have less knowledge about anthrax, a slightly more of them vaccinate their herds than goat farmers (Figure 6). According to the scenario of field, the Upazilla Veterinary Hospitals are distributing anthrax and other vaccines to the farmers free of cost under some projects (NATP-2 & LDDP). So the cattle farmers are getting the vaccine done due to more producer’s group created for cattle in LDDP project rather than for goat.

The common practice for the farmers round the country is to get vaccines from quack (Figure 7). They get the local men always when they call. Though the main source of vaccine is Upazilla Veterinary Hospital, but for administration, the farmers are more dependent on quack. The number of farmers using this source is 25.4% in cattle and 21.33% in goat. Few of the cattle and goat farmers use the staff of Upazilla Veterinary Hospital to vaccinate their herds.

It was seen that 10.61% cattle and 9.33% goat farmers experienced unusual events after anthrax vaccine administration out of the animals vaccinated (Figure 8).

Only 0.32% of cattle farmers and 1.63% of goat farmers experienced death of animals due to anthrax in last 6 months (Figure 9).

CHAPTER-VI

CONCLUSIONS

It is possible to conclude that the vaccine delivery route should be reorganized through proportionate dose allocation based on animal population. Again, the factors discovered to be associated with reduced anthrax vaccination consumption should be addressed by education, advocacy, communication, and social mobilization.

CHAPTER-VII

RECOMMENDATIONS AND FUTURE PERSPECTIVE

The findings of the study recommended the following points:

i) Due to limited doses of anthrax vaccines produced in contrary to the demand (eg. number of animals), the distribution practice may be changed on the basis of anthrax prone areas.

ii) More community engagement program needed to let people know about zoonosis of anthrax as the mass people are illiterate in study area (only 46.3% people of Meherpur are educated).

iii) Further countrywide study needed to know more about the factors influencing the intake of anthrax vaccine in livestock in Bangladesh.

References

Adone R, Rosanna S, Michela F, Massimiliano I, Michela D, Adelia F, Antonio 2016. Development of a sterne-based complement fixation test to monitor the humoral response induced by Anthrax vaccines', Frontiers in Microbiology, 7(JAN), pp. 1–7.

Ahmed BN, Sultana Y, Fatema DS, Ara K, Begum N, Mostanzid SM, Jubayer S. 2010. Anthrax: an emerging zoonotic disease in Bangladesh. Bangladesh Journal of Medical Microbiology. 4(1):46-50.

Ahsan MM, Khan MM, Rahman MF, Hassan MB, Chowdhury J, Parvej SMZH, Jahan MS, Nazir M,Hussain KHMN 2013. Investigation into Bacillus anthracis spore in soil and analysis of environmental parameters related to repeated anthrax outbreak in Sirajganj, Bangladesh. Thai Journal of Veterinary Medicine, 43(3), pp. 449–454.

Chakraborty A,Uddin KS, Hasnat MA, Islam PS, Mikolon MSA, Chakraborty RK, Ahmed BN, Ara KH, Zaki N, Sherif R, Hoffmaster AR, Rahman M, Luby S,Hossain P, Jahangir M 2012. Anthrax outbreaks in Bangladesh, 2009-2010. American Journal of Tropical Medicine and Hygiene, 86(4), pp. 703–710.

Datta S, Sabet K, Mojgan N, Kim P,Valdez L, Patricia A, Gonzalez N, Jose M, Islam S, Mihajlov I, Fierer J, Insel P, Webster A, Nicholas J, Guiney D, Raz GE 2010. Mucosal adjuvant activity of cholera toxin requires Th17 cells and protects against inhalation anthrax. Proceedings of the National Academy of Sciences of the United States of America, 107(23), pp. 10638–10643.

Fasanella A, Garofolo G, Hossain MJ, Shamsuddin M, Blackburn JK, Hugh-Jones M 2013. Bangladesh anthrax outbreaks are probably caused by contaminated livestock feed. Epidemiology and Infection, 141(5), pp. 1021–1028.

Galante D, Manzulli V, Serrecchia L, Di TP, Hugh-Jones M, Hossain MJ, Rondinone V, Cipolletta D, Pace L, Iatarola M, Tolve F, Aceti A, Poppa E, Fasanella A 2021. Investigation on anthrax in bangladesh during the outbreaks of 2011 and definition of the epidemiological correlations. Pathogens, 10(4), pp. 1–10.

FBI Response to Report by Independent Expert Behavioral Analysis Panel on 2001 Anthrax Letters Recent National Press Releases.

General M and Swartz MN. 2001. 'Recognition and Management of Anthrax-An Update', English Journal, 345(22), pp. 1621–1626.

Hugh-Jones M. and Blackburn J 2009. 'The ecology of Bacillus anthracis', Molecular Aspects of Medicine, 30(6), pp. 356–367.

Islam MS, Hossain MJ, Mikolon A, Parveen S, Khan MSU, Haider N, Chakraborty A, Titu AMN, Rahman MW, Sazzad HMS, Rahman M, Gurley ES, Luby SP 2013. Risk practices for animal and human anthrax in Bangladesh: an exploratory study. Infection Ecology & Epidemiology, 3(1), p. 21356.

Joshua JK, Bornaa CS, Seidu B, Jin Z 2020. Mathematical Analysis of the Effects of Controls on the Transmission Dynamics of Anthrax in Both Animal and Human Populations. Alexandria Engineering Journal, 59(6), pp. 5069–5078.

Kaur M, Singh S, Bhatnagar R 2013. Anthrax vaccines: Present status and future prospects. Expert Review of Vaccines, 12(8), pp. 955–970.

Kracalik IT, Kenu E, Ayamdooh EN, Allegye-Cudjoe E, Polkuu PN, Frimpong JA, Nyarko KM, Bower WA, Traxler R, Blackburn JK 2017. Modeling the environmental suitability of anthrax in Ghana and estimating populations at risk: Implications for vaccination and control. PLoS Neglected Tropical Diseases, 11(10), pp. 1–17.

Mongoh MN, Dyer NW, Stoltenow CL, Khaitsa ML 2008. Risk factors associated with anthrax outbreak in animals in North Dakota, 2005: A retrospective case-control study. Public Health Reports, 123(3), pp. 352–359.

Roy PR, Rashid MM, Ferdoush MJ, Dipti M, Chowdury MGA, Mostofa MG, Roy SK, Khan MAHNA, Hossain MM 2014. Biochemical and immunological characterization of anthrax spore vaccine in goat. Bangladesh Journal of Veterinary Medicine, 11(2), pp. 151–157.

Rume FI, Karim MR, Ahsan CR, Yasmin M, Biswas PK 2020. Risk factors for bovine anthrax in Bangladesh, 2010-2014: A case-control study. Epidemiology and Infection. doi: 10.1017/S0950268820000576.

'Sample size Calculator.pdf' (no date). Available at: https://www.calculator.net/sample-size-calculator.html?type=1&cl=95&ci=5&pp=50&ps=69&x=59&y=17.

Sarker M, Shahjahan A, El Z, Mohamed E, Ahosanul HSM, Sarker MA, Rahman MB, Järhult JD, Nazmul Hussain Nazir KHM 2020. Maximization of livestock anthrax vaccination coverage in bangladesh: An alternative approach. Vaccines, 8(3), pp. 1–12.

Thouret JM, Rogeaux O, Beaudouin E, Levast M, Ramisse V, Biot FV, Valade E, Thibault F, Gorgé O, Tournier JN 2020. Case report of an injectional anthrax in france, 2012. Microorganisms, 8(7), pp. 6–10.

'WHO_CDS_VPH_93.117 very important document the south african control measure.pdf' (1992).

Thouret JM, Rogeaux O, Beaudouin E, Levast M, Ramisse V, Biot FV, Valade E, Thibault F, Gorgé O, Tournier JN 2004. Risk factors for human anthrax among contacts of anthrax-infected livestock in Kazakhstan. American Journal of Tropical Medicine and Hygiene, pp 48-52.

Appendix A: Photo Gallery

Fig: Discussion on anthrax surveillance in Gangni with respected DLO, Meherpur

Fig: Data collection from cattle farmer

Fig: Data collection from goat farmer

Fig: Awareness, communication and social mobilization (ACSM)

Fig: A cured cutaneous anthrax case (Farmer)

Fig: Treatment given to this calf against suspected anthrax

Appendix B: Questionnaire for LRI Head

Questionnaire (LRI Head, Dhaka & In charge, LRI Cumilla)

Interviewer ID-……

Date- ……/……./……

Part-A: General Information			
Sl. No.	**Question**	**Response**	**Go to**
A1	Name of institution	☐LRI Mohakhali ☐LRI Cumilla	
A2	Name of interviewee		
A3	Designation		
A4	Mobile number		
A5	How long have you been posted in the current job(production & distribution)?	……..Months/Years	
Part-B: Vaccine production & distribution related information			
B1	What is the maximum yearly capacity of anthrax vaccine production at your facilities?	…….doses	
B2	How many dose you produce in last five years?	2022……..; 2021……;2020…….. 2019……..;2018……..doses	
B3	How many dose you distribute produce in last five years?	2022……..; 2021……;2020…….. 2019……..;2018……..doses	
B4	Leftover produced doses of vaccine(if any)?	2022……..; 2021……;2020…….. 2019……..;2018……..doses	
B5	Which are the divisions do your facility cover for vaccine distribution?	…… *(Division names)*	
B6	If no, how do you manage?		
B7	What is the strategy to decide vaccine dose allotment in your covered area?	Please tick (√) one: ☐District demand ☐ Own target met up ☐ Both ☐Others(specify)…….	
B8	If the answer is 'District demand' or 'Both' who send you the demand?	Please tick (√) one: ☐Divisional Director ☐ DLO ☐ ULO ☐Others(specify)…….	
B9	What is the frequency of distribution?	Please tick (√) one: ☐Weekly ☐Once in 15 days ☐ Monthly ☐Others	

B10	Which Division/District demand highest anthrax vaccine?	Please mention 1. 2. 3.	
B11	Which season(s) there's highest demand of anthrax vaccine?		
B12	Why?		
B13	Do you have mandate to provide extra anthrax vaccine to special District?	☐ Yes ☐ No	
B14	If yes, which District? Why?		
B15	How do you deliver the vaccines to destination?	Please tick (√) one: ☐Cool Van ☐Ice packed courier ☐Messenger with ice box ☐ Messenger without ice box ☐Others(specify)……..	
B16	Do you have any retail sale center for vaccine?	☐Yes ☐No	
B17	Do you distribute vaccine directly from LRI to livestock owner?	☐ Yes ☐ No	
B18	Do you have any vaccine distribution Standard Operating Procedure (SOP)?	☐ Yes ☐ No	
B19	Do you have any recommendation to develop production and distribution of anthrax vaccine?		

Appendix C: Questionnaire for DD, DLO, ULO

Questionnaire (DD/DLO)

Interviewer ID-……

Date- ……/……./……

Part-A: General Information			
Sl. No.	**Question**	**Response**	**Go to**
A1	Name of office		
A2	Name of interviewee		
A3	Designation		
A4	Mobile number		
A5	How long have you been posted in the current job?	……..Months/Years	
Part-B: Vaccine production & distribution related information			
B1	What is the maximum yearly demand of anthrax vaccine in your Division/District/Upazilla?	2022……..; 2021……;2020…….. 2019……..;2018……..doses	
B2	Do you get anthrax vaccine according to demand?	☐ Yes ☐ No	If yes, skip B3
B3	If no, how do you manage?		
B4	How do you prepare demand of anthrax vaccine?	Please tick (√) one/multiple: ☐APA target base ☐Livestock population base☐ Both ☐Others	
B5	How do you get the vaccine demand from District/Upazilla level?	Please tick (√) one/multiple: ☐Through letter ☐Over phone ☐Others	
B6	Do you have mandate to provide extra anthrax vaccine to special district/Upazilla?	☐ Yes ☐ No	
B7	If yes, which district/Upazilla? Why?		
B8	How do you receive vaccine at Division/District	Please tick (√) one/multiple: ☐Through LRI Vehicle ☐Own messenger ☐Both ☐Others	

	level?		
B9	What is the frequency of vaccine distribution?	Please tick (√) one: ☐Weekly ☐Once in 15 days ☐ Monthly ☐Others	
B10	Do you provide ice pack with vaccine?	☐ Yes ☐ No	
B11	How do you distribute vaccine to field level?	Please tick (√) one/multiple: ☐Through office vehicle ☐Messenger from field office ☐Both ☐Others	
B12	Do you think that the existing channel of vaccine distribution is working well?	☐ Yes ☐ No	
B13	Do you have any recommendation to develop production and distribution of anthrax vaccine?		
Factors affecting distribution of anthrax vaccine (Objective-2 for ULO)			
C1	Do you store vaccine at your office?	☐ Yes ☐ No	
C2	If yes, do you have refrigerator?	☐ Yes ☐ No	
C3	Is there enough space for vaccine storage?	☐ Yes ☐ No	
C4	If no, how do you store vaccines?	Please tick (√) one: ☐Use other refrigerator ☐Keeping vaccine in cool box with ice ☐ Both ☐Others	
C5	Did you hear about anthrax outbreak in any of your District/Upazilla in last one year?	☐ Yes ☐ No	
C6	If yes, what did you do?		

Appendix D: Questionnaire for SALO, LSP, CEAL and Union Vaccinator

Questionnaire (SALO/CEAL/LSP/VACCINATOR)

Interviewer ID-……	Date- ……/……./……

Part-A: General Information			
Sl. No.	**Question**	**Response**	**Go to**
A1	Name of office		
A2	Name of interviewee		
A3	Designation		
A4	Mobile number		
A5	How long have you been engaged with current job?	……..Months/Years	
Part-B: Vaccine distribution related information			
B1	Do you vaccinate livestock?	☐ Yes ☐ No	
B2	How do you get anthrax vaccine?	Please tick (√) one: ☐From DLO office ☐ From ULO office☐From LRI directly ☐ Specify if others……	
B3	How the farmers finds you?	Please tick (√) one: ☐They come to office ☐ They call over phone☐I use to go door to door ☐ Others	
B4	Do the farmers bring livestock at office?	☐ Yes ☐ No	
B5	If no, do you go door to door?	☐ Yes ☐ No	
B6	How do you vaccinate livestock?	Please tick (√) one/multiple: ☐Through campaign ☐Door to door ☐ Farmers bring livestock to office ☐All	
B7	Do you carry ice pack with vaccine while distribution?	☐ Yes ☐ No	
B8	If yes, is that sufficient?	☐ Yes ☐ No	
B9	What is the frequency of vaccine distribution?	Please tick (√) one/multiple: ☐Daily ☐Weekly ☐Fortnightly ☐ Monthly ☐Others (specify)	
B10	Is the vaccine doses sufficient to meet the demand?	☐ Yes ☐ No	

Part-C: Factors affecting distribution of anthrax vaccine (Objective-2)			
C1	Which animal(s) do you give anthrax vaccine?	Please tick (√) one/multiple: ☐Cattle ☐Goat ☐ Buffalo ☐Sheep ☐Others	
C2	If you give anthrax vaccine to goat and or sheep, why?	☐ Act as potential career ☐ Dose is half ☐ Easy to administer ☐ Others (specify)…….	
C3	If you don't give anthrax vaccine to goat and or sheep, why?	☐ Irritation after vaccination ☐ Shock and death ☐ Owners disagree ☐ Others (specify)…….	
C4	Did you hear about anthrax outbreak in your Upazilla in last one year?	☐ Yes ☐ No	
C5	When you go for vaccination?	Please tick (√) one/multiple: ☐Early morning ☐Noon ☐ Afternoon Evening	
C6	Could you finish a 100 dose vial at one visit?	☐ Yes ☐ No	
C7	If no, what do you do with that?		
C8	Do you have enough time to vaccinate properly beyond office work?	☐ Yes ☐ No	
C9	Do you have any recommendation to improve anthrax vaccination activity in your area?		

Appendix E: Questionnaire for farmers

Questionnaire

Interviewer ID-……

Date- ……/……./……

Part-A: General Information			
Sl. No.	**Question**	**Response**	**Go to**
A1	Farm ID		
A2	Farmer's name		
A3	Age of farmer (years)		
A4	Gender of the owner	Please tick (√) one: ☐ Male……1 ☐ Female……..2 ☐ Others……....3	
A5	Religion	Please tick (√) one: ☐ Islam……..1 ☐ Hindu……2 ☐ Christian……3 ☐ Buddhist……4	
A6	Address	District……..Upazilla…….. Union……Ward…….. Village…….....	
A7	Farm Location(Geo Location)	Latitude……..... Longitude……	
A8	Primary occupation of the owner-	Please tick (√) one: ☐ Farmer(Poultry/Crops/Fish)…1 ☐ Fisherman…….....2 ☐ Student(Specify)……3 ☐ Businessman……..4 ☐ Religious Leader/Imam…….5 ☐ Construction Worker……..6 ☐ Rickshaw/Van Puller……..7 ☐ Street Vendor……..8 ☐ Day Laborer……9 ☐ Government Service ……..10 ☐ Teacher……11 ☐ Other(Specify……)…..12	
A9	What is the education level of farm owner?	Please tick (√) one: ☐ No Schooling……1 ☐ Primary School (<= Grade 5)…….2 ☐ High School (Grade 6-10)……...3 ☐ Higher Secondary (Grade 11-12)….4 ☐ Tertiary Level (Grade >12)……..5 ☐ Madrasah……..6 ☐ Vocational……7 ☐ Others(Specify……)……..8	

A10	Contact Number-		
A11	What type of farm is it?	Please tick (√) one: ☐Cattle farm……..(no.) ☐Goat farm…… ☐Sheep farm…… ☐Others(specify)……	
A12	Purpose of farming?	Please tick (√) one: ☐Milk ☐Meat ☐Mixed ☐Others(specify)	
A13	How long have you been engaged with farming?	……..Months/Years	
Part-B: Disease related information			
B1	Do you know about anthrax?	☐ Yes ☐ No	
B2	If yes, from where did you hear about anthrax?	Please tick (√) one or more: ☐Neighbour……1 ☐Local market ……2 ☐ Upazilla Veterinary Hospital……...3 ☐ Quack ……...4 ☐ TV/Newspaper ……5 ☐ Others ……..6	
B3	To which species, anthrax occurs?	Please tick (√) one or more: ☐Cattle ☐Goat ☐Buffalo ☐Human ☐All species	
B4	How many times anthrax occurred in last one year?		
B5	Do you know about anthrax signs in livestock?	☐ Yes ☐ No	
B6	If yes, what happened if anthrax occurs in livestock?	Please note the comments 1. 2. 3.	
B7	Do you know about anthrax signs in human?	☐ Yes ☐ No	
B8	What happened if anthrax occurs in human?	Please note the comments 1. 2. 3.	
B9	Have you seen anthrax spread?	☐ Yes ☐ No	
B10	If yes, how it spreads?	Please note the comments 1. 2. 3.	
Part-C: Factors affecting low intake of anthrax vaccine			
C1	Do you know about anthrax vaccine?	☐ Yes ☐ No	

C2	If yes, how do you know?	Please tick (√) one: ☐ Upazilla Veterinary Hospital……1 ☐ Quack…….2 ☐ Pharmacy……...3 ☐ Others(specify)…….….4	
C3	What are the benefits to give vaccine?	Please note the comments 1. 2. 3.	
C4	Do you get vaccine when you want?	☐ Yes ☐ No	
C4	If no, why?	Please note the comments 1. 2. 3.	
C5	Have you given vaccine to your livestock against anthrax?	☐ Yes ☐ No	
C6	If yes, why?		
C7	If no, why?		
C8	What is the source of anthrax vaccine?	Please tick (√) one: ☐ Upazilla Veterinary Hospital……1 ☐ Quack…….2 ☐ Pharmacy……...3 ☐ Others(specify)…….….4	
C9	Did you find anything abnormal happened after vaccination to animal?	☐ Yes ☐ No	
C10	If yes, to which species and what's wrong?	Please note the comments 1. 2. 3.	
C9	How much you have to pay for vaccination per animal?	Govt staff ……tk/animal Quack ……...tk/animal Vaccinate in campaign…….tk/animal	
C10	In which season do you give anthrax vaccine to your animals?	Please tick (√) one: ☐ Monsoon or pre/post monsoon…….1 ☐ Summer or pre/post summer……..2	

		☐ Winter or pre/post winter……...3 ☐ Spring or pre/post spring …….4 ☐ Not season specific…….5	
C11	What is the frequency of vaccination?	Please tick (√) one: ☐ Bi-annually ☐ Annually ☐ Don't know	
C12	Did you get any benefit from vaccination?	☐Yes ☐ No	
C13	If yes, what are the benefits?		
C14	Do you see any anomalies during vaccination?		Do not use ice; Do not use proper dose etc.
C15	Do you have any opinion on how to make vaccination program more successful?		
D. Risk Factors related to Anthrax			
D1	Do you send the animals to green pasture?	☐Yes ☐ No	
D2	Do you feed water hyacinths to your animal?	☐Yes ☐ No	
D3	Do you see abandoned fly around your farm?	☐Yes ☐ No	
D4	Have you faced any animal death in last 6 months due to anthrax?	☐Yes ☐ No	
D5	If yes, what did you do with the dead animals?		

Brief Biodata of the Author

Dr. Sudeb Sarker had passed the Secondary School Certificate Examination in 2004 followed by Higher Secondary Certificate Examination in 2006. He obtained his DVM Degree in 2011 from Chattogram Veterinary and Animal Sciences University (CVASU). Now, he is a candidate for the degree of Masters in Applied Veterinary Epidemiology under the One Health Institute, CVASU. He has immense interest to continue research on AMR and infectious disease epidemiology through One Health approach.