Project ID 321

Competitive Research Grant

Sub-Project Completion Report

on

Seroprevalence and identification of associated risk factors of Q-fever (*Coxiellaburnetii*) in ruminants; an emerging zoonotic disease in Bangladesh

Project Duration
May 2016 to September 2018

Department of Pathology and Parasitology
Patuakhali Science and Technology University
Khanpura, Babugonaj, Barishal-8210

Submitted to
Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Seroprevalence and identification of associated risk factors of Q-fever (*Coxiellaburnetii*) in ruminants; an emerging zoonotic disease in Bangladesh

Project Implementation Unit
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Bangladesh Agricultural Research Council (BARC)
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Project Implementation Unit
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New Airport Road, Farmgate, Dhaka – 1215
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Acknowledgement

The execution of CRG sub-project has successfully been completed by Patuakhali Science and Technology University using the research grant of USAID Trust Fund and GoB through Ministry of Agriculture. We would like to thanks to the World Bank for arranging the grant fund and supervising the CRGs by BARC. It is worthwhile to mention the cooperation and quick responses of PIU-BARC, NATP 2, in respect of field implementation of the sub-project in multiple sites. Preparing the project completion report required to contact a number of persons for collection of information and processing of research data. Without the help of those persons, the preparation of this document could not be made possible. All of them, who made it possible, deserve thanks. Our thanks are due to the Director PIU-BARC, NATP 2 and his team who have given their whole hearted support to prepare this document. We hope this publication would be helpful to the agricultural scientists of the country for designing their future research projects in order to technology generation as well as increasing production and productivity for sustainable food and nutrition security in Bangladesh. It would also assist the policy makers of the agricultural sub-sectors for setting their future research directions.

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Table of Contents

List of Tables

List of Figures

Executive Summary

The objective of this study was to know the prevalence rate and confirmation of the presence of Q-fever in cattle and goats in some selected districts in Bangladesh. In this investigation, Chuadanga and Jhenaidah districts were selected for goat sample collection and Sirajgonj and Pabna districts were selected for cattle sample collection. Randomly collected 252 blood sera (goat-84, cattle-168) and 252 bulk milk (goat-84, cattle-168) samples of goats and cattle were tested by indirect ELISA (iELISA). The positive cut-off value of iELISA in the individual blood sera and in the bulk milk were ≥ 40 and ≥ 30 respectively. The overall seropositivity of Q-fever in goats and cattle was 11.9% ($P < 0.05$) and 9.5% ($P < 0.05$) respectively. Milk-positivity of Q-fever in goats and cattle was 10.7% ($P < 0.05$) and 8.3% ($P < 0.05$) respectively. The prevalence of Q-fever in goats was higher in Chuadanga district than in Jhenaidah district. The prevalence of Q-fever in cattle was higher in Sirajgonj district than in Pabna district. The prevalence of tick infestation in cattle was 42.0% and 46.6% for male and female respectively. In goats the prevalence was found to be 45.5% and 52.1% in male and female respectively. The prevalence of abortion in tick infested animals was 16.4% in cattle and 15.8% in goats. The seroprevalence of *coxiella butnetii*, the organism responsible for Q-fever was relatively higher in female (11.02% and 12.33%) than in male (6.00% and 9.09%) both for cattle and goats respectively. In this investigation 2 samples of goat (2.38%) and 2 samples of cattle (1.19%) were positive for *C. burnetii* infection on polymerase chain reaction (PCR), out of 84 goat and 168 cattle whole blood samples respectively. It was revealed from this study that the disease Q-fever is present in goats and cattle in Bangladesh.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. **Title of the CRG sub-project:**
   Seroprevalence and identification of associated risk factors of Q-fever (*Coxiella burnetii*) in ruminants; an emerging zoonotic disease in Bangladesh

2. **Implementing organization:** Patuakhali Science and Technology University

3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**

Principal Investigator:
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4. **Sub-project budget (Tk):**
   4.1 Total: Tk 28, 04, 260.00 (Twenty eight lac four thousand two hundred sixty only)
   4.2 Revised (if any):

5. **Duration of the sub-project:**
   5.1 Start date (based on LoA signed): 17 May 2017
   5.2 End date : 30 September 2018

6. **Justification of undertaking the sub-project:**

Coxiella burnetii is an obligate intracellular zoonotic bacterium that causes Q-fever, an emerging disease in Bangladesh (Rahman *et al.*, 2016; Haider *et al.*, 2015). Ticks are considered as the natural primary reservoir of *C. burnetii* and are responsible for spread of the infection in wild as well as domestic animals (Norlander, 2000). Hard ticks *Hyalomma anatolicum* and *Rhipicephalus sanguineus* are vectors for this organism and are common in Bangladesh (Ahmed *et al.*, 2007). In animals, the organism is mainly found in the reproduction system and may primarily cause abortion or infertility (Porter *et al.*, 2011). Thus, *C. burnetii* infection has a significant economic impact on animal production (Porter *et al.*, 2011).

A relationship between *C. burnetii* infection with age and sex has also found in animals, particularly in cattle. Several studies have shown that the prevalence of *C. burnetii* infection increases with age or with the number of parity in cattle and sheep (Bottcher *et al.*, 2011; Garcia-Ispierto *et al.*, 2011; Kennerman *et al*., 2010; McCaughey *et al.*, 2010; Paul *et al*., 2014). Several studies in cattle show that seroprevalence increases with an increasing in herd size (McCaughey *et al*., 2010; Paul *et al*., 2012). Several management factors such as housing system, isolation of newly introduced animal, etc. may also contribute to seroprevalence of *C. burnetii* infection in animals (Capuano *et al*., 2001; Paul *et al.*, 2012).

Infected animals contaminate the environment by shedding the organism in milk, feces, urine, saliva and large quantities in birth fluid and placenta (Porter *et al.*, 2011). The bacteria remain infective for months in aerosol and contaminated dust (Woldehiwet, 2004). Contact with infected livestock or being in the vicinity of infected livestock have been identified as significant risk factors for human exposure or disease (van Der Hoek *et al.,* 2011). Antigenic variations of *C. burnetii* usually exhibits phase I in chronic and Phase II in acute form of disease (Pierre-Edouard and Didier, 1999). At higher risk for the disease are veterinarians, and slaughter house workers, particularly those working with ruminants (Marrie and Fraser, 1985).

7. Sub-project goal:

Determination of seroprevalence and identification of risk factors for *Coxiella burnetii* in ruminants (Cattle and goats) in Bangladesh.

8. Sub-project objective (s):

i) To determine the seroprevalence of Q-fever in ruminants in some selected areas of Bangladesh.
ii) To isolate and identify *Coxiella burnetii* DNA from seropositive samples
iii) To determine the associated risk factors for Q-fever in the selected areas

9. Implementing location (s):

For cattle samples: Sirajgonj and Pabna Districts
For goat samples: Chuadanga and Jhenaidah Districts

10. Methodology in brief:

The study was carried out at the Department of Pathology and Parasitology, Patuakhali Science and Technology University, Babugonj, Barishal.

10.1. Determination of seroprevalence of Q-fever in ruminants

Collection of Samples

A total of 252 blood samples of animals (goat 84 and cattle 168) were collected which had or had not the history of reproductive problem such as abortion, retention of placenta, etc. and also from male goats and cattle. Summary of the sample collection is shown in Table 1. In this study, Chuadanga and Jhenaidah districts were selected for goat samples and Sirajgonj and Pabna districts were selected for cattle samples. Species, age, sex and history of abortion, retention of placenta and tick infestation were recorded for each of the samples as reported by the animal owners as per structured questionnaire. The blood samples were collected into sterile tubes maintaining sterile conditions and the sera separated in the laboratory according to the standard procedures. The samples were brought to the laboratory maintaining cold chain and centrifuged for 10 minutes at 4000 rpm to separate the sera. The sera were stored at -20 ^{0}C until use. A total of 252 (goat 84 and cattle 168) milk samples were centrifuged and the nonfat fraction was stored at -20 ^{0}C until tested for antibodies against *C. burnetii.* Project activities at the field are shown in Figure 1.

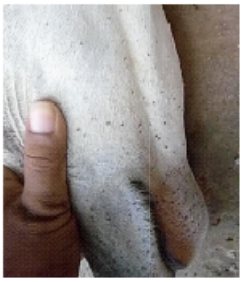
Table 1. Summary of the sample collection from Sirajgonj, Pabna, Chuadanga and Jhenaidah districts

Areas	Types of samples	No. of samples	Remarks
Sirajgonj District	Cattle Blood	Male:25, Female:59	Blood samples (Cattle): 168 Blood samples (Goat): 84 Milk samples (Cattle): 168 Milk samples (Goat): 84
	Cattle Milk	Female:84	
Pabna District	Cattle Blood	Male:25, Female:59	
	Cattle Milk	Female: 84	
Chuadanga District	Goat Blood	Male:6, Female:36	
	Goat Milk	Female:42	
Jhenaidah District	Goat Blood	Male:5, Female:37	
	Goat Milk	Female:42	

Figure 1. Project activities at the field

Collection of cattle blood samples from Sirajgonj and Pabna districts

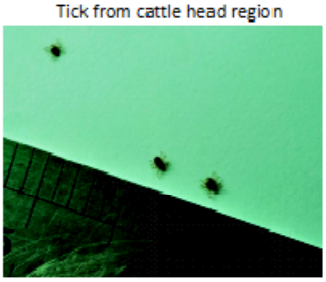
Collection of goat blood samples from Jhenaidah and Chuadanga districts



Tick infestation in cattle

Tick from cattle head region

Tick infestation in goat ear



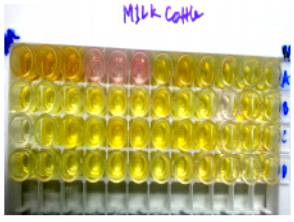
Tick from goat ear

Tick infestation in cattle and goat and isolated ticks

Serological assay

Antibodies against *C. burnetii*, both in the blood serum and in the bulk milk were detected using indirect enzyme-linked immunosorbant assay (iELISA) using the Bovine Anti-Q-fever antibody ELISA kit and Goat Q-fever ELISA kit (My Biosource, Inc., San Diego, California, USA. #MBS1602362, #MBS018309), according to manufacturer's instructions. All reagents were kept into 18-26 ^{0}C until use. Before use, the reagents were mixed by shaking gently. Every test was conducted with the positive and negative control sera supplied with the kit. Results were expressed as a percent value of the test sample optical density (OD%) which was calculated as OD% = [(OD sample – OD negative control) / (OD positive control – OD negative control) × 100]. The positive cut-off value of iELISA in the individual blood sera and in the bulk milk were ≥ 40 and ≥ 30 respectively.

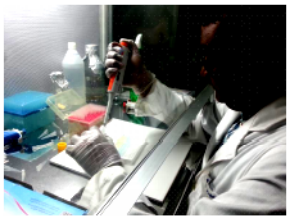
Figure 2. Activities showing ELISA test

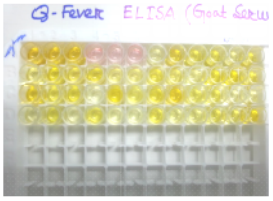


Cattle Milk-ELISA

Reading by ELISA reader

Q-fever ELISA KIT (Cattle)





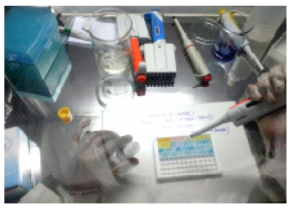
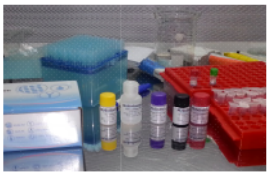
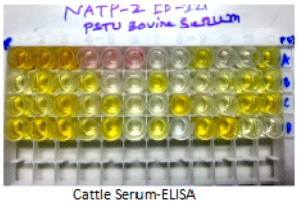


Fig: 4- Goat Serum-ELISA (First 3 well Positive Control, next 3 well negative control and others wells are test serum samples



Q-fever ELISA KIT (Goat)



Cattle Serum-ELISA

10.2. Isolation and identification of *Coxiella burnetii* DNA

Genomic DNA from seropositive samples of *C. burnetii* was extracted from the whole blood using a commercial PureLink™ Genomic DNA Mini Kit (Invirogen, Van Allen Way Carlsbad, CA, USA. # K 1820-02) according to the manufacturer's instructions. The extracted DNA was stored at -20 ^{0}C until use. The PCR Master Mix (Promega, Madison, USA) was used for PCR amplification. In this PCR, primer sets, used for amplification of the species-specific transposon repetitive region of *C. burnetii*, were Trans 1: 5´- TGG TAT TCT TGC CGA TGA C-3´and Trans 2: 5´- GAT CGT AAC TGC TTA ATA AAC CG-3´ with the amplicon size of 687 bp (Houver *et al.*, 1992 and Kılıçet *et al.* 2016). Amplified PCR products were run on a 1.5% agarose gel for electrophoresis under 100 volts for 2 hours. Following electrophoresis, the gel was stained using ethidium bromide (0.5 µg/ml) or Safe-White and results were evaluated by UV transillumination.

Figure 3. Activities showing genomic DNA isolation from seropositive samples of *C. burnetii*

Genomic DNA extraction kit

Whole bood samples from cattle and goats

Genomic DNA purification was done from whole blood of cattle and goat by PureLink™ Genomic DNA Kit (Invitrogen) in National Institute of Biotechnology(NIB) Lab.

10.3. Determination of the associated risk factors for Q-fever

For determining the risk factors associated with Q-fever in the areas under study a survey was conducted among the participating farmers using a structured questionnaire as shown below.

Questionnaire of Q-fever survey

PERSONAL DETAILS: **Date:**

Name: …… **Age:** …….. **Sex**: □ Male □ Female

Address: ……..

Phone number: +8801

Species name: ……. **Age:** …… **Sex**: □ Male □ Female

Pregnancy:□ Yes □ No □Not applicable

---

1. Have you heard of Q-fever?

□ Yes □ No □ Don`t know

2. Have you been vaccinated against Q-fever?

□ Yes □ No □ **Don`t know**

3. Have you been diagnosed with Q-fever?

□ Yes □ No □ **Don`t know**

4. Have you been tested prior to vaccination and found to be exposed?

□ Yes □ No □ **Don`t know**

5. Would you consider consulting your doctor for Q-fever screening and diagnosis?

□ Yes □ No □ Not applicable (already vaccinated)

6. If not vaccinated, can you give a reason?

□ Not considered important □ Did not know about it □ Too costly

□ Unable to get vaccinated □ Others

7. Have you spent time in any of the following fields (secondary job, work-experience, volunteer)?
Note: more than one answer can be chosen.

□ Abattoir □ Veterinary □ Companion animal breeding

□ Wildlife care □ Animal shelter □ Farm worker/manager

□ Pet shop □ Other

8. Have you ever assisted an animal give birth?

□ Yes □ No

9. What type of animal did you assist (more than one answer possible)

i. ii. iii. iv. v.

10. Did you wear any personal protective equipment?

□ Gloves □ Mask □ Apron □ Gumboots □ Others

11. **Have you consumed raw (unpasteurized) milk?**

□ Yes □ No

12. If you live or work on a farm, do you run a feedlot?

□ Yes □ No □ Not applicable

13. If you live or work on a farm, do you run a dairy enterprise?

□ Yes □ No □ Not applicable

14. If you live or work on a farm, can you give a brief description of the enterprise (size, type and number of animals, type of activities).
Mention:

15. Have you ever seen ticks on your livestock?

□ Yes □ No

If yes, **do you use any method of control?**

16. Do you home-slaughter stock or assist in the process?

□ Yes □ No

17. If you manage a property, have you introduced new stock in the past 12 months?

□ Yes □ No □ Not applicable

18. Do you ageist stock on your property?

□ Yes □ No □ Not applicable

19. Do you lend stock to other producers that are then bought back to your property?

□ Yes □ No □ Not applicable

20. Do you see feral or pest animals on your property

□ Yes □ No □ Not applicable

If yes Please specify (fox, rabbit, pig, wild dog, goat, etc.) –

21. If you breed stock on your property, have you identified an increased abortion or stillbirth rate in the past?

□ Yes □ No □ Not applicable

22. If yes to above, was a veterinarian consulted?

□ Yes □ No □ Not applicable

23. If yes, was a diagnosis reached?

□ Yes □ No □ Not applicable

What was the diagnosis?

24. Is the property where you live or work within 5 km of a town with more than 9000 people?

□ Yes □ No □ Not applicable

Statistical analysis

The chi-square test was used to analyze difference among the groups. A p-value of < 0.05 was considered statistically significant. The analytical software package GraphPad Prism version 5.0 (GraphPad Software Inc., La Jolla, CA, USA) was used for the statistical analysis.

11. Results and discussion:

11.1. Determination of seroprevalence of Q-fever in ruminants

Prevalence of tick infestation

A total number of 168 cattle (male 50, female 118) and 84 goats (Male 11, female 73) were observed for tick infestation**.** The prevalence of tick infestation was 42.0% in male cattle and 46.6% in female cattle. On the other hand prevalence of tick infestation was 45.5% in male goats and 52.1% in female goats (Table 2). Noor *et al.*, 2016 reported the rate of tick infestation in Black Bengal goats to be 53.33%, which is close to our findings.

Table 2. Prevalence of tick infestation in cattle and goats

Species		No. of observed animals	No. of tick infested animals	Prevalence (%)	χ2 Test *P* value
Cattle	Male	50	21	42.0	<0.05
	Female	118	55	46.6	<0.05
Goat	Male	11	05	45.5	<0.05
	Female	73	38	52.1	<0.05

Prevalence of abortion in animals

A total of 118 female cattle and 73 female goats were examined for the history of abortion. The prevalence of abortion was 15.3% in female cattle and 13.7% in female goats (Table 3).

Table 3. Prevalence of abortion in animals

Species	No. of observed animals	No. of abortion	Prevalence (%)	χ2 Test *P* value
Cattle	118	18	15.3	<0.05
Goat	73	10	13.7	<0.05

Prevalence of abortion in tick infested animals

A total number of 55 female cattle and 38 female goat samples were examined for history of both tick infestation and abortion. The prevalence was 16.4% in cattle and 15.8% in goats (Table 4).

Table 4. Prevalence of abortion in tick infested animals

Species	No. of tick infested animals	No. of abortion	Prevalence (%)	χ2 Test *P* value
Cattle	55	09	16.4	<0.05
Goat	38	06	15.8	<0.05

Prevalence of retention of placenta in animals

A total number of 118 female cattle and 73 female goats were examined for the history of retention of placenta. The prevalence was 9.3% in cattle and 8.2 % in goats (Table 5).

Table 5. Prevalence of retention of placenta in animals

Species	No. of observed animals	No. of placenta retained	Prevalence (%)	χ2 Test *P* value
Cattle	118	11	9.3	<0.05
Goat	73	06	8.2	<0.05

Prevalence of retention of placenta in tick infested animals

A total number of 55 female cattle and 38 female goats were examined for the history of both tick infestation and retention of placenta. The prevalence was 12.7% in cattle and 10.5% in goats (Table 6).

Table 6. Prevalence of retention of placenta in tick infested animals

Species	No. of tick infested animals	No. of placenta retained	Prevalence (%)	χ2 Test *P* value
Cattle	55	07	12.7	<0.05
Goat	38	04	10.5	<0.05

Q-fever vaccination status in animals

A total number of 168 cattle and 84 goats were observed for the history of Q-fever vaccination. There was no report of Q-fever vaccination in cattle and goats.

Seroprevalence of-Q fever in cattle and goats

In this study animal level seroprevalence of Q-fever and the herd level prevalence of Q-fever in cattle and goats based on bulk milk were estimated by using indirect ELISA test. The overall seroprevalence of Q-fever in goats and cattle from the study area were 11.9% (***P*** < 0.05) and 9.5% (***P***

< 0.05) respectively (Table 7) indicating that Q-fever is an existing disease in ruminants of Bangladesh. According to Rahman *et al*., 2018 in Bangladesh, the prevalence of the disease in animal was 6.97% also, by Rahman *et al.,* 2016 and Haider *et al*., 2015, the prevalence of the disease was 6.38% and 0.7% (7/1149) in ruminants. Epidemiological investigations mainly rely on serological tools due to the lack of cardinal signs of the disease. Therefore, ELISA was chosen to detect Q-fever seroprevalence in animals for its cheap rate and the safety (Rousset *et al.,* 2010). The overall prevalence of Q-fever in bulk cow milk and goat milk were 8.3% (***P*** <0.05) and 10.7% (***P*** <0.05) respectively (Table 8). Other researchers Rahman *et al.,* 2016 and IO *et al*., 2014 reported a herd level (bulk milk) prevalence of Q-fever to be 15.6% and 24.2% respectively in dairy cattle. This variation in prevalence might be due to variation in age, sex, environmental condition, geographical distribution, atmosphere, time, rainfall, herd size, housing system, species, etc. Using serum ELISA test sex-wise prevalence of Q-fever was found to be 6% and 11% in male and female cattle respectively whereas in goats the prevalence was 9% and 12% respectively in male and female (Table 9).

Table-7. Prevalence of seropositivity of Q-fever in goats and cattle

Species	Tested serum samples	ELISA positive	Prevalence	χ2 Test *P* value
Goat	84	10	11.9%	<0.05
Cattle	168	16	9.5%	<0.05

Table 8. Prevalence of milk-positivity of Q-fever in goats and cattle

Species	Tested milk samples	ELISA positive	Prevalence	χ2 Test *P* value
Goat	84	09	10.7%	<0.05
Cattle	168	14	8.3%	<0.05

Table 9. Sex-wise seroprevalence of Q-fever in cattle and goats

Species	Total No. of animals	Serum-ELISA positive	χ2 Test *P* value
Male cattle	50	3 (6%)	
Female cattle	118	13 (11.02%)	<0.05
Male goat	11	1 (9.09%)	
Female goat	73	9 (12.33%)	<0.05

District-wise prevalence of Q-fever in cattle and goats

District-wise prevalence of Q-fever using blood serum and milk from cattle and goats is shown in Figures 4, 5, 6 and 7. ELISA with goat serum revealed the prevalence of Q-fever in goats to be (6 out of 42) 14.3% (***P*** < 0.05) and (4 out of 42) 9.5% (***P*** < 0.05) in Chuadanga and Jhenaidah district respectively. Likewise, ELISA with cattle serum revealed the prevalence of Q-fever to be (10 out of 84) 11.9% (***P*** < 0.05) and (6 out of 84) 7.1% (***P*** < 0.05) in Sirajgonj and Pabna district respectively. When ELISA was performed using milk the prevalence for goats, was found in Chuadanga and Jhenaidah district to be (6 out of 42) 14.3% (***P*** < 0.05) and (3 out of 42) 7.14% (***P*** < 0.05) respectively and for cattle the prevalence in Sirajgonj and Pabna district was (9 out of 84) 10.7% (***P*** < 0.05) and (5 out of 84) 6.00% (***P*** < 0.05) respectively.

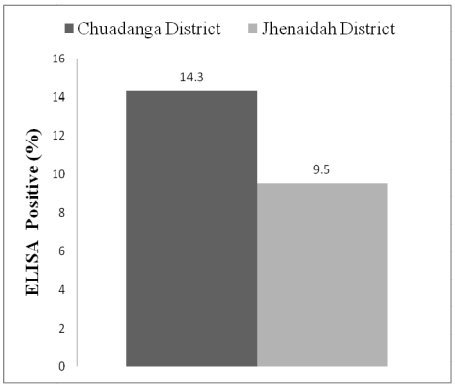


Figure 4. District-wise prevalence of Q-fever using Goat serum-ELISA

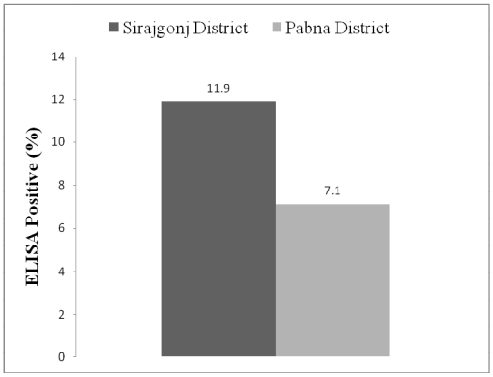


Figure 5. District-wise prevalence of Q-fever using Cattle serum-ELISA

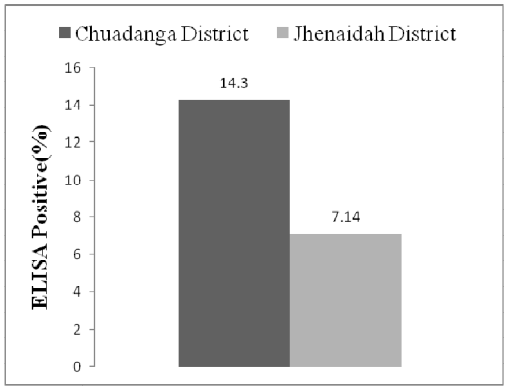


Figure 6. District-wise prevalence of Q-fever using Goat milk-ELISA

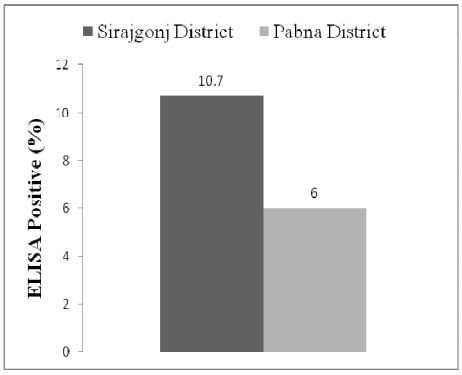


Figure 7. District-wise prevalence of Q-fever using Cattle milk-ELISA

11.2. Isolation and identification of *Coxiella burnetii* DNA

Presence of *C. burnetii* in the blood samples of cattle and goats was confirmed by PCR. The PCR products were examined for specificity using 1.5% agarose gel electrophoresis (Figure 8). According to polymerase chain reaction (PCR) analysis the prevalence of *C. burnetii* infection was only 1.19% and 2.38% in cattle and goats respectively (Table 10). Rahman *et al*., 2018 reported that only one tick (0.79%) was *C. burnetii* positive through real time PCR analysis. Rahman *et al*., 2016 found only one sheep placenta to be *C. burnetii* positive in real time PCR. Eibach *et al*., 2012 reported that blood samples of 8% of goats and 3% of sheep were PCR positive for *C. burnetii.* Kirkan *et al*., 2008 reported that a total of 6 (4.3%) cattle serum samples were PCR positive for *Coxiella burnetii.*

Table 10. Genomic DNA purification and amplification by PCR

Species	No. of samples (Whole blood)	DNA purification (Seropositive samples)	PCR positive for *C. brunetii*
Cattle	168	16	2 (1.19%)
Goat	84	10	2 (2.38%)

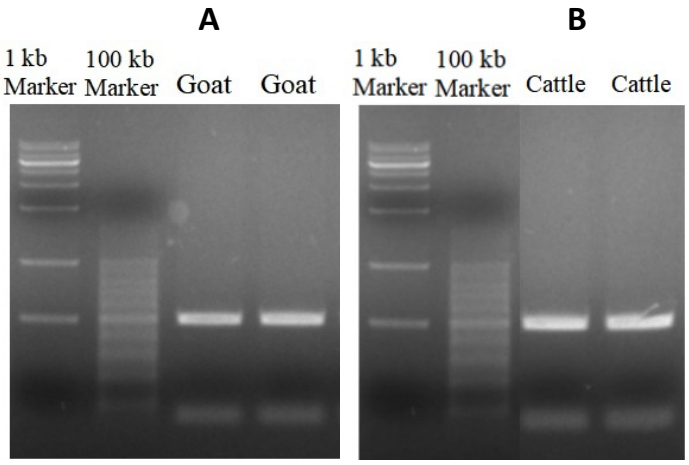


Figure 8. Agarose gel electrophoresis of PCR product

A) Represents goat blood samples; From left: lane 1 and lane 2 DNA marker, lane 3 and lane 4 PCR product (687 bp) from goat blood samples. B) Represents cattle blood samples; From left: lane 1 and lane 2 DNA marker, lane 3 and lane 4 PCR product (687 bp) from cattle blood samples.

11.3. Determination of the associated risk factors for Q-fever

Due to time constraints it was not possible to conduct a detailed study for determination of the risk factors associated with Q-fever in the country however, concentration of tick population, age of the animals, housing system, sex differences, seasonal variation, grazing condition, high human and animal population density, close contact between animals and human, etc. might have played a significant role in determining the risk factors for Q-fever.

According to the result of serum-ELISA and the milk-ELISA prevalence of Q-fever in goat at Chuadanga and Jhenaidah districts was 14.3% (***P*** <0.05), 14.3% (***P*** <0.05) and 9.5% (***P*** <0.05), 7.14% (***P*** <0.05) respectively (Figures 4 and 6). This variation might be due to more tick population at Chuadanga than Jhenaidah district. According to the result of serum-ELISA and the milk-ELISA prevalence of Q-fever in cattle at Sirajgonj and Pabna district was 11.9% (***P*** <0.05), 10.7% (***P*** <0.05)

and 7.1% (***P*** <0.05), 6.0% (***P*** <0.05) respectively (Figures 5 and 7). This variation might be due to more loose housing system at the Sirajgonj district than Pabna district. The sex wise prevalence of Q-fever was 6% in male cattle, 11.02% (***P*** <0.05) in female cattle, 9.09% in male goats and 12.33% (***P*** <0.05) in female goats (Table 9). The prevalence of Q-fever was higher in female than in male animals. Seo *et al.*, 2017 also reported that prevalence of Q-fever was higher in female cattle (12.9%) than in male cattle (1.3%). In this case hormonal differences might have played role in determining the susceptibility to infection. The herds under study were originated from major dairy milk pockets of Bangladesh like Sirajgong and Pabna districts. Goat milk was collected from Jheniahdah and Chaudanga districts. Dairy cattle are usually chronically infected with Q-fever and shed *C. burnetii* in the milk (Lang, 1989). The cattle management system in Sirajgonj area slightly varies from that of other parts of Bangladesh. In the dry season, the cattle graze freely and remain in pasture ("Bathan") for almost six months (December to May). As a result, a lot of intermixing among cattle of different owners occurs during that period. Intermixing of cattle from different owners may facilitate the transmission of infection in dairy cattle herd of this area. Dry environmental conditions during that period (December to May) could have played a significant role in the survival of the bacteria and thus facilitating the transmission between animals. Similarly, higher prevalence of Q-fever in loose housing system was also reported by Paul *et al*., 2012. The risk of infection coincides with the time the host reaches adulthood (Muskens *et al*., 2011) and adult cattle are exposed to *C. burnetii* and tick bites for extended periods (Guatteo *et al*., 2011). In the world, Bangladesh has one of the highest human and animal population densities with 1000 persons (World Bank, 2007) and 145 domestic ruminants per square kilometer (Bangladesh Agricultural Research council, 2010: Haider *et al*., 2014). The human-animal density and frequent contact between animals and humans make the country an ideal site for Q-fever and other zoonotic diseases to emerge. In Bangladesh, rural people generally have both very close contact to and occupational involvement with cattle and goats providing an opportunity for Q-fever transmission.

Evidence of Q fever in Bangladesh has important implications for livestock production and public health and warrants further investigation. Veterinarians in Bangladesh should consider Q-fever as a differential diagnosis when dealing reproductive disorders in cattle and goats. Physicians, who routinely deal with unexplained human cases of febrile illness, hepatitis, meningoencephalitis, and/or endocarditis, should consider Q-fever as a differential diagnosis.

12. Research highlight/findings:

- The overall seropositivity of Q-fever in goats and cattle was 11.9% and 9.5% respectively.
- Milk-positivity of Q-fever in goats and cattle was 10.7% and 8.3% respectively.
- The prevalence of tick infestation was 42% & 45.5% in male and 46.6% & 51.7% in female both for cattle and goats respectively.
- The prevalence of abortion in tick infested animal was 16.4% in cattle and 15.8% in goats.
- The seroprevalence of Q-fever was higher in female (11.02% & 12.33%) than in male (6.00% & 9.09%) both for cattle and goats respectively.
- On polymerase chain reaction (PCR), out of 84 goat samples 2 (2.38%) and 168 cattle samples 2 (1.19%) were positive for *C. burnetii* infection.

B. Implementation Position

1. Procurement

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment	Laser printer (1)	10000	Laser printer (1)	10000	
(b) Lab &field equipment	**GD1**		**GD1**		100% achievement
	a) ELISA reader (1)	350000	a) ELISA reader (1)	350000	
	b) Centrifuge machine	54000	b)Centrifuge machine	54000	
	(1)	20000	(1)	20000	
	c) Microwave oven(1)	10000	c) Microwave oven (1)	10000	
	d) Ice box(1)		d) Ice box (1)		
	GD2	100000	**GD2**	100000	
	a) PCR kit or	25000	a) PCR kit or	25000	
	equivalent (2)		equivalent (2)		
	b) PCR primers (5)	10000	b) PCR primers (5)	10000	
	c) Agarose		c) Agarose		
	(electrophoresis		(electrophoresis		
	grade) (1)	6000	grade) (1)	6000	
	d) PCR tubes 0.2ml		d) PCR tubes 0.2ml		
	(eppendorf or		(eppendorf or		
	equivalent) (1)	15750	equivalent) (1)	15750	
	e) Disposables tips for	10000	e) Disposables tips for	10000	
	Micropipettes of	10000	Micropipettes of	10000	
	different grades (21)	1000	different grades (21)	1000	
	f) Ethidium bromide(1)	20000	f) Ethidium bromide(1)	20000	
	g) PBS solution(2)	10000	g) PBS solution(2)	10000	
	h) Spirit(2)	6000	h) Spirit(2)	6000	
	i) Vacutainer tube(4)	2500	i) Vacutainer tube(4)	2500	
	j) Tris-Hcl(1)		j) Tris-Hcl(1)		
	k) Eppendrof tube(2)	2000	k) Eppendrof tube(2)	2000	
	l) Test tube(50)	2000	l) Test tube(50)	2000	
	m) Eppendrof tube		m) Eppendrof tube		
	plastic rack (2)		plastic rack (2)		
	n) Conical flask(5)		n) Conical flask(5)		
	GD3	20000	**GD3**	20000	
	a) Micropipette sets:	20000	a) Micropipette sets:	20000	
	Single channel:	20000	**Single channel:**	20000	
	200 -1000 µl ,(1)	20000	200 -1000 µl ,(1)	20000	
	50 -300 µl,(1)		50 -300 µl,(1)		
	10 -50 µl,(1)	30000	10 -50 µl,(1)	30000	
	0.5 -10 µl(1)	30000	0.5 -10 µl(1)	30000	
	b) **Multichannel:**	200000	b) **Multichannel:**	200000	
	50-300 µl,(1)	100000	50-300 µl,(1)	100000	
	10 -50 µl9(1)		10 -50 µl9(1)		
	c) Indirect ELISA kit(2)	30000	c) Indirect ELISA kit(2)	30000	
	d) Genomic DNA		d) Genomic DNA		

	extraction kit(2) e) DNA sequencing (3)		extraction kit(2) e) DNA sequencing (3)		
(c) Other capital items	i)-20 °C medical refrigerator (12 CFT) (1) ii) Gel documentation System (1) iii) Electrophoresis set (1) 1v) Thermo cycler (1)	100000 100000 50000 350000	i)-20 °C medical refrigerator (12 CFT) (1) ii) Gel documentation System (1) iii) Electrophoresis set (1) 1v) Thermo cycler (1)	100000 100000 50000 350000	

2. Establishment/renovation facilities:

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	
					There was no such provision in the budget

3. Training/study tour/ seminar/workshop/conference organized: None

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	-	-	-	-	There was no such provision in the budget
(b) Workshop	-	-	-	-	

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	615880	615880	615880	0	100%	
B. Field research/lab expenses and supplies	1179250	1113981	1113981	0	94.47%	
C. Operating expenses	205180	174251	173166	1085	84.40%	
D. Vehicle hire and fuel, oil & maintenance	70000	69491	69491	0	99.27%	
E. Training/workshop/seminar etc.	0	0	0	0	0	
F. Publications and printing	75000	58450	12450	0	16.6%	46000 refunded
G. Miscellaneous	49000	31620	31620	0	64.53%	
H. Capital expenses	609950	585802	585802	0	96.04%	

N.B: Tk 46000.00 was refunded to Director, PIU-BARC, NATP-2 through crossed cheque

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
i) To determine the seroprevalence of Q-fever in ruminants in some selected areas of Bangladesh	i) Cattle farm selection at Sirajgong and Pabna districts ii) Goat farm selection at Chuadanga and Jhenaidah districts iii) Blood collection, serum preparation iv) Determination of seropositivity of Q-fever using ELISA Kit v) Milk sample collection from cattle and goat and determination of milk positivity of Q-fever using ELISA Kit	• The overall seropositivity of Q-fever in goats and cattle was 11.9% and 9.5% respectively. • Milk-positivity of Q-fever in goats and cattle was 10.7% and 8.3% respectively. • The prevalence of tick infestation was 42.0%, 45.5% in male and 46.6%, 52.1% in female both for cattle and goats respectively. • The prevalence of abortion in tick infested animal was 16.4% in cattle and 15.8% in female goats. • The seroprevalence of Q-fever was higher in female (11.02%, 12.33%) than in male (6.00%, 9.09%) both for cattle and goat respectively.	i) Awareness about Q-fever in farmers. ii) Farmers will be benefited from proper management and treatment of Q-fever specially in aborted animals.
ii) To isolate and identify *Coxiella burnetii* DNA from seropositive samples	i) Genomic DNA extraction from whole blood samples of cattle and goats. ii) Amplification of DNA using PCR. iii) Analysis of PCR products on a 1.5% agarose gel and analysis of the PCR products by UV transillumination.	On polymerase chain reaction (PCR) 2.38% goats and 1.19% cattle were found positive for *C. burnetii* infection.	Confirmatory diagnosis of *C. burnetii* using PCR will help proper diagnosis of the disease in goats and cattle in Bangladesh that in turn will help controlling Q-fever.
iv) To determine the associated risk factors for Q-fever in the selected areas	i) Development of questionnaires for field survey on Q-fever. ii) Conducting survey and risk factor analysis on the history of age, gender, seasons, species, environment, housing systems, grazing facilities, etc.	i) Female animals were more prone to Q-fever than male animals ii) Goats were more prone to Q-fever than cattle	i) Management of animals on the basis of identified risk factors will help farmers to avoid infection with Q-fever.

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer, etc.	-	-	
Journal publication	02	-	
Information development	-	-	
Other publications: Scientific Conference	-	01	Seroprevalene and identification of associated risk factors of Q-fever (*Coxiella burnetii*) in ruminants as an emerging disease in Bangladesh; The Coastal Vet Society Bangladesh 2nd Annual Scientific Conference 2019

F. Technology/Knowledge generation/Policy Support (as applied):

i. **Generation of technology (Commodity & Non-commodity)**

Polymerase chain reaction (PCR) has been adopted for the identification *Coxiella burnetii,* the organism responsible for Q-fever.

ii. **Generation of new knowledge that help in developing more technology in future**

It is revealed from this study that Q-fever is present in cattle and goats in Bangladesh.

iii. Technology transferred that help increased agricultural productivity and farmers' income

None

iv. Policy Support

The knowledge of the presence of Q-fever in cattle and goats in Bangladesh will help policy makers in developing proper prevention, control and treatment measures to combat Q-fever in ruminants in Bangladesh.

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring (description & output of consultation meeting, monitoring workshops/seminars etc.): None

ii) Field Monitoring (time & No. of visit, Team visit and output):

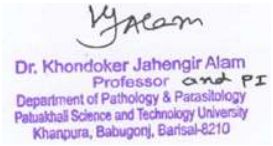
Monitoring team	Date(s) of visit	Total visit till date (No.)	Remarks
PIU-BARC, NATP-2	20/02/18	01	
Technical Division/ Unit, BARC	28/03/18	01	
Internal Monitoring	05/04/18, 26/04/18, 10/05/18	03	

I. Lesson Learned (if any)

Nothing particular

J. Challenges (if any)

To keep pace with time was a challenge as the time allocated for this study was too short.



Signature of the Principal Investigator
Date …….
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Counter signature of the Head of the organization/authorized representative
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