

Full Length Research Paper

Molecular epidemiology of *Coxiella burnetii* in human, animals and ticks in Bangladesh

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Q fever, caused by *Coxiella burnetii* is a well recognised zoonotic disease around the world which is endemic in many countries. In Bangladesh, a very few information is available in men and animals about this disease. Thus, the objective of this study is to determine the presence of Q fever in animal (goat and cattle) and human population including the tick which is a vector of the disease. A total of 172 animals with abortion and reproductive disorder history were collected from Shahjadpur Upazilla of Sirajgonj district in Bangladesh. 150 sera samples from patients of Mymensingh Medical College Hospital were collected which had a history of pyrexia. 127 tick samples were collected from the body of animals randomly. The samples were tested with CHEKIT Q-Fever antibody Enzyme Linked Immunosorbent Assay (ELISA) Test Kit (indirect ELISA) primarily and the positive sera were retested by real time polymerase chain reaction (PCR) to detect DNA of *C. burnetii*. Indirect ELISA revealed the prevalence result of 6.97 and 0% in animals and humans, respectively and only one tick was Reverse transcription Polymerase Chain Reaction (RT-PCR) positive (0.79%). It is thus observed that this is the first time the presence of *C. burnetii* in ticks using real time PCR is reported.

Key words: *Coxiella burnetii*, Enzyme Linked Immunosorbent Assay (ELISA) antibody, polymerase chain reaction (PCR), sera samples.

INTRODUCTION

Q fever is a disease which is zoonotic in nature caused by *Coxiella burnetii*. Cattle, sheep and goat act as a

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primary reservoir host of this disease (Arricau-Bouvery and Rodolakis, 2005; McCaughey et al., 2011) and has also been found in other mammals like birds, fish, reptiles, ticks and other arthropods (Porter et al., 2011). The personnel related with livestock (farmers, veterinarians, abattoir workers, laboratory personnel working with *Coxiella burnetii*) are at higher risk for Q fever (Maurin and Raoult, 1999). Wild and domestic mammals serve as reservoirs and are part of the bacterium's life cycle. The environment is contaminated by vaginal secretions, placenta, milk, feces, urine, saliva and other by-products of infected animals (Porter et al., 2011). *C. burnetii* is transmitted mainly by inhalation or by contact with infectious tissues, although tick-transmission of this bacterium has also been reported (Rolain et al., 2005). Human infections with *C. burnetii* are usually transmitted by aerosol during contact with infected animal feces or bird residues (Tissot-Dupont et al., 2004). Abortion, infertility and other reproductive problems are the main problems in animal due to Q fever (To et al., 1998). Therefore, significant economic losses occurred when a farm is infected with Q fever (Porter et al., 2011). In humans, it affects the respiratory system, musculoskeletal system, digestive system, nervous system and cardiovascular system (Karakousis et al., 2006).

The disease has been well documented and reported in many countries of the world (Porter et al., 2011; McCaughey et al., 2011; Arricau-Bouvery and Rodolakis, 2005; Maurin and Raoult, 1999). Haider et al. (2015) and Rahman et al. (2016) reported the presence of Q fever in Bangladesh. In Bangladesh, animal and human density is quite high (World Bank, 2007) and they mostly share the same premises which facilitates the transmission of Q fever and other emerging zoonotic diseases from animals to humans and vice versa. Rahman et al. (2016) reported the prevalence of Q-fever in domestic ruminants by serological tests and immunoglobulin G of *C. burnetii* was detected by Haider et al. (2015) with a prevalence of 0.7% (8/1149) in the ruminants, specifically 0.65% (4/620) in cattle, and 0.76% (4/529) in goats by using enzyme linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA).

The limitations of the studies on Q fever in Bangladesh cited above are; this study used only a small number of samples from abortion positive herds and others only from sick animals admitted in the hospitals. Serological tests only, were used without credible information about the sensitivity and specificity of the tests. Moreover, there is no information regarding the status of *C. burnetii* in humans and ticks. A systemic scientific study using molecular technique is essential for characterization of *C. burnetii* in Bangladesh.

MATERIALS AND METHODS

The study was carried out at the Zoonotic Disease Diagnostic Laboratory, Medicine Department, Faculty of Veterinary Science, BAU, Mymensingh, Bangladesh with the collaboration of OIE Reference Laboratory for Q fever, Institute of Bacterial Infections and Zoonoses, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institute, Naumburger Str. 96a, 07743 Jena, Germany. The study was done through the following steps below;

Collection of samples

A total of 172 sera samples of animals (goat and cattle) were collected which had the history of reproductive problem such as anestrus, abortion, retention of placenta etc. from Shahjadpur Upazilla of Sirajgonj district in Bangladesh. 5ml of blood were collected from each animal with a 10 ml sterile syringe. The serum was made by keeping it undisturbed for about 10 h. The supernatant was collected and centrifuged. Each serum was collected separately in a sterile screw capped tube using micropipette and tube was labeled.

Blood sample from 150 patients with the history of pyrexia of unknown origin was performed with the help of physicians from Mymensingh Medical College Hospital and each serum was collected and stored at -20°C until further use. Samplings were performed with respect to human consent and animal welfare.

A total of 127 ticks were examined carefully by hand picking in other to keep the mouth part intact. Collected ticks were kept in vials with necessary examination and identification done in the laboratory according to the standard method, following the keys and description given by Soulsby (1962).

Serologic surveillance of the diseases

All reagents were mixed by gentle shaking at 18 to 26°C. Samples were checked twice and their optical densities (OD) were averaged. The OD of negative control was corrected by subtracting it. CHEKIT Q-Fever antibody ELISA Test Kit (IDEXX, Liebefeld-Bern, Switzerland) was used to test the sera (Gwida et al., 2014).

DNA extraction and real time polymerase chain reaction (PCR)

The tick was crushed with mortar and pestle and DNA was extracted (Schmoock et al., 2014). High pure polymerase chain reaction (PCR) Template Preparation Kit™ (Roche Diagnostics, Mannheim, Germany) was used according to the instructions of the manufacturer. With a Taq Man based real-time PCR, assay was performed for DNA quantification targeting the transposase element (Klee et al., 2006).

The real-time PCR assay was performed with the primers and the conditions as described in Chakrabarti et al. (2016). Samples were considered positive with a cycle threshold (Ct) < 40 (Boarbi et al., 2014).

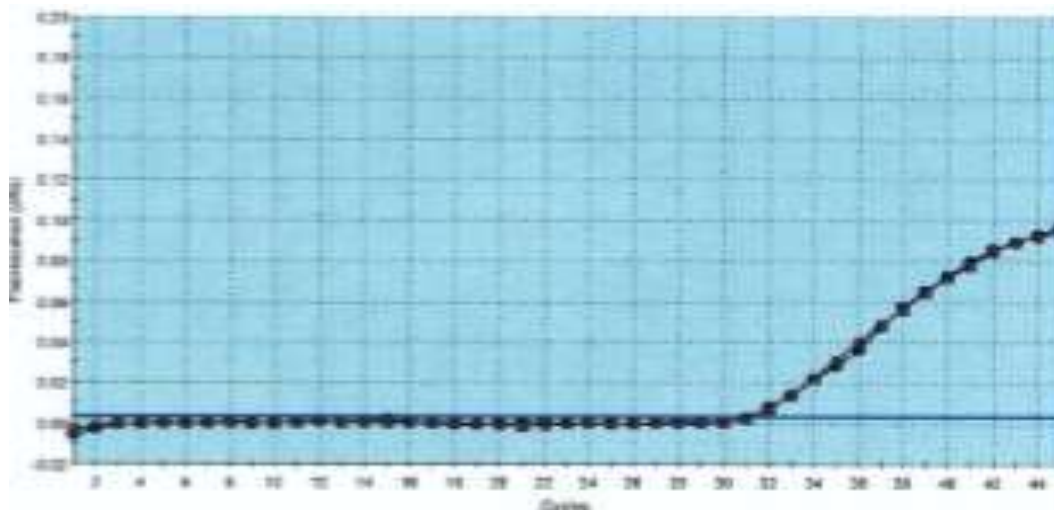
Statistical analysis

Data were entered into MS excel 2007. Cleaning and processing of data were performed then transferred to Specific Statistical software

Table 1. Prevalences of *C. burnetii* in animals, humans and ticks.

Species	Tested	Positive ELISA	Prevalence (%)	PCR
Animal	172	12	6.97	Negative
Human	150	0	0	ND
Tick	127	ND	0.79	Positive

ND, Not done.

**Figure 1.** Real time PCR amplification plots for DNA extracted from ticks of Bangladesh. The ct-results for the positive tick samples are amplified.

for further analysis. Double entry was done to assure the quality of the data obtained. Statistical Package for Social Services (SPSS) version 20.0 was used for analysis.

RESULTS AND DISCUSSION

The overall seropositivity of *C. burnetii* antibodies were 6.97% in animal and 0% in humans and no positive seropositive sera was found by real time PCR (Table 1).

C. burnetii was detected in only one tick (0.79%) through real time PCR (Figure 1). In this study, it was observed that seroprevalences of *C. burnetii* antibodies were higher in animal which is same as other authors (Khalili and Sakhaee, 2009; McQuiston and Childs, 2002). According to Rahman et al. (2016) in Bangladesh, the prevalence of the disease in ruminant was 6.38% also, by Haider et al. (2015) the prevalence of the disease was 0.7% (8/1149) in ruminants (0.65% in cattle and 0.76% in goats). There were no positive results in

humans using indirect ELISA in this study and one tick was found real time PCR positive.

Normally, the diagnosis of Q fever is done by serological test, culture of the organism and conventional polymerase chain reaction (PCR). The pathogen isolation is the best method for proper diagnosis, but working with this organism is hazardous for laboratory workers and thus, level 3 biosafety cabinets are required for it (Arricau-Bouvery and Rodolakis, 2005; Fournier et al., 1998). 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 man and animals for its cheap rate and safety level (Rousset et al., 2010).

Samples from these areas where low or subclinical coxiellosis present only serological test may not be specific and sensitive enough for the diagnosis of *C. burnetii* infection due to cross-reactions (Rousset et al., 2009). Moreover, seroconversion occurs after 3 to 4 weeks post infection in time frame which is a limitation of

serological diagnostic methods. In addition, antibodies against *C. burnetii* often appears late in the course of the disease, therefore it is difficult to diagnose in early stage only by serological test in animals. Although, early diagnosis is necessary to treat the affected one. DNA-based method is the best method for early diagnosis. Methods such as PCR, nested PCR and real-time PCR have successfully been used for clinical identification of *C. burnetii* (Kato et al., 1988; Berri et al., 2000; Klee et al., 2006).

In this study, there were no seropositive cases in humans and none of these seropositive samples of the animal was positive in real time PCR. Other authors had also reported positive value in ELISA tested sera sample but all real time PCR were negative (Khaled et al., 2012). Due to large population of human (above 16 crores) and animal in Bangladesh (23.4 million cattle, 1.86 million buffaloes, 33.5 million goats, and 1.1 million sheep), the zoonotic diseases spread very fast. Since Q fever is a zoonotic disease and social living pattern of Bangladesh which allows intermixing of species, it can be an outbreak any time anywhere.

Conclusion

This study thus proves the prevalence of Q fever in animals in Bangladesh which is observably the first time the presence of *C. burnetii* in ticks using real time PCR is reported. Further research should be conducted by collecting the animal and human sample from the same geographical area for better understanding of the transmission of disease between them and actual prevalence on that area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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