Bangl. J. Vet. Med. (2016). 14 (1): 65-68 ISSN: 1729-7893 (Print), 2308-0922 (Online)

PREVALENCE OF *COXIELLA BURNETII* INFECTION IN CATTLE, BLACK BENGAL GOATS AND TICKS IN BANGLADESH

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ABSTRACT

The objectives of this study were to determine the prevalence of *Coxiella burnetii* infection in domestic ruminants and to detect *Coxiella burnetii* DNA from ticks and serum samples. A total of 24 ticks, 91 goats and 81 cattle serum samples with the history of abortion and reproductive disorders were collected from the different areas in Bangladesh. The serum samples were tested by CHEKIT Q-Fever Antibody ELISA Test Kit and *Coxiella burnetii* DNA was detected by multiplex quantitative real-time PCR. The overall prevalence was 7.6% and 6.1% in goats and cattle, respectively. However, none of seropositive samples and tick samples was positive in quantitative real-time PCR.

Keywords: Q fever, *Coxiella burnetii*, Black Bengal goat, cattle, tick, Bangladesh

INTRODUCTION

Q fever is a widespread zoonosis that is caused by *Coxiella burnetii*, an obligate, Gram-negative, intracellular bacterium (Baca and Pratesky, 1983; Behymer and Riemann, 1989; Arricau-Bouvery *et al.*, 2005; Kazar, 2005). This disease, described for the first time among abattoir workers in 1937 in Queensland, Australia, is now recognized as being endemic worldwide (Derrick, 1983; Maurin and Raoult, 1999). Both public and animal health issues are closely related to Q fever. The reservoir includes mammals, birds and arthropods mainly ticks (Ioannou *et al.*, 2009). Domestic ruminants are often asymptomatic carriers of *C. burnetii* and are considered the most important reservoir and source for human Q fever (Woldehiwet, 2004). *Coxiella burnetii* is present in clinically inconspicuous sheep flocks (Angela *et al*., 2012). However, other animal species like birds, reptiles, arthropods or pets can also be infected and possibly transmit the disease to humans (Angelakis and Raoult, 2009). In humans, Q fever can lead to an acute disease (self-limited febrile illness, pneumonia, or hepatitis) or to a chronic disease, mainly endocarditis in immunocompromised patients or patients suffering from valvulopathy and abortions and stillbirth in pregnant women (Angelakis and Raoult, 2009). Q fever or coxiellosis occurs during late pregnancy (about 15 days before term) and in animals provide abortions and stillbirths in goats and sheep, infertility, mastitis and endometritis in cattle (Heinzen *et al.*, 1999; Woldehiwet, 2004; Arricau-Bouvery *et al.*, 2005; Berri *et al.*, 2005) with associated economic impact for the herd.

To control the spread of *C. burnetii* from animals to humans, the detection of shedders of *C. burnetii* and the knowledge of the prevalence of the infection are imperative. Only few reports on the prevalence of Q fever in animals are available in Bangladesh on the prevalence of Q fever in domestic ruminants were found from literature search in Bangladesh (Haider *et al*., 2015; Rahman *et al*., 2016). Haider *et al*. (2015) reported seroprevalence of Q fever in cattle and goats that were brought to veterinary hospitals in Netrokona, Dinajpur, and Chittagong districts in Bangladesh. Rahman *et al*. (2016) reported herd-level prevalence of Q fever in dairy cattle investigating bulk milk (Satkhira, Rajshahi, Chittagong), animal-level seroprevalence in cattle, goats and sheep originating from herds (Mymensingh, Sherpur, Dhaka) with previous history of abortion and detection of *Coxiella burnetii* DNA from sheep placenta. No report on seroprevalence of Q fever in cattle and black Bengal goats from Pabna, Sirajganj, Kurigram districts of Bangladesh reported in literature. Moreover, the prevalence of *C. burnetii* infection in ticks was not reported from Bangladesh. The objectives of this study were to determine the prevalence of *C. burnetii* infection in cattle and black Bengal goats in selected districts and to detect *C. burnetii* DNA from ticks and serum samples.

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MATERIALS AND METHODS

The study was conducted in Kurigram Sadar, Shahjadpur, Pabna Sadar, Mymensingh Sadar during December, 2014 to December, 2015. Blood samples and ticks were collected from cattle and black Bengal goats and then sent from Bangladesh to Germany by world courier service (in dry ice) following recommended sample transportation rules of IATA for serology and real-time PCR. Cows and black Bengal goats with the history of reproductive disorders (retained fetal membrane, abortion, anestrous, repeated breeding) were selected purposively for blood sampling. Five to seven milliliter of blood was drawn from the jugular vein using 10 ml sterile syringe and kept in slant position at room temperature for 12 h. After clotting, the syringe with blood was kept overnight at 2-8°C. The serum was separated using micropipette in sterile screw capped tubes, labeled and stored at -20°C until further use.

The ticks were handpicked from randomly selected cattle and goats and preserved at -20C. The ticks were identified morphologically with the aid of a photographic microscope using tick identification keys (Beati and Keirans, 2001; Walker *et al.*, 2003).

The sera were tested by iELISA for *C. burnetii* antibodies according to the procedure described by Gwida *et al.* (2014). All reagents were brought to 18-26°C before use. The reagents were mixed by shaking gently. All samples were tested in duplicates (twice) and the optical densities (OD) of the samples were averaged and corrected by subtracting the OD of the negative control. Serum based tests were performed using the commercial CHEKIT Q-Fever Antibody ELISA Test Kit (IDEXX, Liebefeld-Bern, Switzerland) based on *C. burnetii* inactivated phase 1 and phase 2 antigens (Paul *et al.*, 2012). The positive cut-off value (S/P ratio) of iELISA individual animal sera was ≥ 40%.

DNA from seropositive samples of *C. burnetii* was extracted according to the method described by Schmoock *et al.* (2014) using the High Pure PCR Template Preparation Kit™ (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. DNA quantification was performed with a TaqMan based real-time PCR assay targeting the transposase element IS1111 as described by Klee *et al.* (2006). The real-time PCR assay was performed using primers and conditions as shown (Table 1 & 2). Samples were considered positive with a cycle threshold (Ct) < 40 (Boarbi *et al.*, 2014).

Table 1. Primers used for IS 1111 Real-time PCR

Oligo	Name	Sequence (5′ to 3′)
Primer	Cox- R	CCCCGAATCTCATTGATCAGC
	Cox- F	GTCTTAAGGTGGGCTGCGTG
Probe	Cox- TM	6FAM-AGCGAACCATTGGTATCGGACGTTXTATGG-PH

Table 2. PCR Conditions

Cycler: Stratagene MX 3000	Temperature	Time
Initial denaturation	50°C	2 min
	95°C	10 min
Denaturation, Cycles 50	95°C	15 sec
Anneal / Elong. Cycles 50	60°C	30 sec

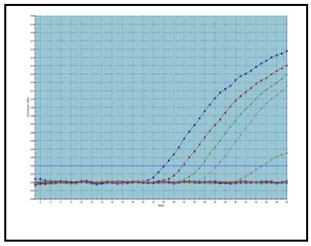

Figure 1. Real time PCR amplification plots from DNA extracted from sera of seropositive cows and black Bengal goats of Bangladesh.

RESULTS AND DISCUSSION

The overall seropositivity was 6.1% in cattle and 7.6 % in goats. None of the seropositive sera and tick samples was positive in quantitative real-time PCR (Table 3 & Figure 1).

Table 3. Prevalence of Q fever in cattle, goats and tick

Species	Tested	ELISA positive	Prevalence	PCR
Cattle	81	05	6.1%	Negative
Goats	91	07	7.6%	Negative
Ticks	24	Not done	-	Negative

The present study indicated a relatively higher seroprevalence of Q fever in goats than in cattle. Other authors had also reported significantly higher seroprevalences of Q fever in goats than in cattle (Khalili and Sakhaee, 2009; McQuiston and Childs, 2002).

In Bangladesh, Rahman *et al*. (2016) reported an overall seroprevalence of Q fever of 6.38% in domestic ruminants originating from herds with a history of abortion whereas, Haider *et al.* (2015) detected immunoglobulin G for *C. burnetii* in 0.7% (8/1149) of ruminants (0.65% (4/620) in cattle and 0.76% (4/529) in goats) by using ELISA and Immunofluorescence assay.

The traditional diagnosis of Q fever is based on culture, serology and conventional PCR. The isolation of the pathogen is a reliable diagnostic method, but it requires level 3 biosafety laboratories t and is hazardous for the laboratory personnel (Fournier *et al.*, 1998; Arricau-Bouvery and Rodolakis, 2005). Since there is no characteristic clinical presentation for Q fever, epidemiological investigations mainly rely on serological tools. Hence, ELISA was found to be the method of choice for Q fever seroprevalence studies in man and animals (Rousset *et al.*, 2010)

The diagnosis of *C. burnetii* infection by some serological tests may not be specific and sensitive due to cross-reactions (Rousset *et al.*, 2009) especially in samples from areas with a low prevalence of coxiellosis. In addition, serological diagnosis is retrospective due to delayed seroconversion (3 to 4 weeks post infection), making serology useless for timely treatment. Early diagnosis is helpful for an early onset of treatment. Thus DNA-based methods such as PCR, nested PCR and real-time PCR have been developed and successfully used for detection of *C. burnetii* in clinical specimens (Kato *et al.*, 1988; Berri *et al.*, 2000; Klee *et al.*, 2006). However, none of seropositive samples and tick samples was positive in real-time PCR. Other authors had also reported sera have a positive value in ELISA test but all the RT-PCR were negatives (Khaled *et al.*, 2012). Only a positive PCR result is significant, a negative result does not rule out Q fever.

This study and previous reports from Bangladesh indicate that Q fever is prevalent in cattle and black Bengal goats in Bangladesh. Q fever is a zoonosis and we suppose that Q fever is also prevalent in humans in Bangladesh. Persons especially those occupationally exposed to animals should be screened for Q-fever regularly.

ACKNOWLEDGEMENTS

The authors are grateful to National Reference Laboratory for Q fever, Friedrich-Loeffler-Institute, Jena, Germany for taking samples from Bangladesh and for travel grant for first author to travel to Germany.

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