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Molecular and serological investigation of *Coxiella burnetii* in goats with reproductive disorders[#]

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Abstract

One of the important yet neglected diseases in goats that affect the reproductive system is Q fever, caused by the Gram-negative, obligate intracellular pathogen, Coxiella burnetii. Molecular techniques cannot be used solely to detect the presence of this pathogen, due to the shedding pattern of the organism. So, both molecular and antibody detection assays were used to know the actual burden of the disease. Although many countries and states have reported Q fever in independent studies, the actual scenario of C. burnetii in Kerala is unknown. In the present study milk, vaginal swabs and blood samples were collected randomly from 150 goats with reproductive disorders in Thrissur, Palakkad and Malappuram districts. The presence of antibodies (7.99 per cent) against C. burnetii revealed the presence of seropositive goats in Kerala. None of the samples were positive by PCR targeting the Com1 gene and *IS1111* genes.

Keywords: *Q fever, Coxiella burnetii, serosurveillance, PCR, ELISA*

A highly contagious zoonotic disease known as coxiellosis or Q fever is caused by the intracellular Gram-negative bacterium *Coxiella burnetii*. Following a severe outbreak in the Netherlands from 2007 to 2010, which resulted in 4000 human cases reported and the culling of 58,150

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goats, this pathogen gained public and scientific attention. ELISA is the most popular and accurate method for Q fever surveillance in the Indian subcontinent. However, due to insufficient diagnostic resources and low levels of knowledge about the disease among the general public, clinicians and veterinarians, it largely continues to be a hidden and neglected zoonosis in India. Hence molecular serological methods were used in this study for the detection of *C. burnetii.*

A total of 450 samples comprising 150 milk, 150 vaginal swabs and 150 blood samples were collected from goats with reproductive disorders from Thrissur, Palakkad and Malappuram districts of Kerala (n=50 each from each district). The DNA was extracted from milk using the phenol-chloroform method and vaginal swab samples using kit (Qiagen blood and tissue kit) method, respectively. The PCR was standardised using a positive control procured from the Division of Veterinary Public Health repository, Indian Council of Agricultural Research- Indian Veterinary Research Institute-(ICAR-IVRI), Bareilly, Uttar Pradesh. By using primers (Table 1) and positive control, PCR was performed in a final volume of 25 µL reaction mixture using 2 µL of standard strain DNA as template. The cycling conditions for PCR of *Com1* and *IS1111* genes are given below (Table 2).

The amplicons for *Com1* and *IS1111* genes were obtained with an expected base pair size of 438 and 687 bp, respectively (Fig.1 and Fig. 2).

None of the DNA samples extracted from milk and vaginal swab of goats harboured *Com1* and *IS1111* gene. In the present study, this finding was supported by Sahu *et al.* (2018)

Table 1. Primers used for identification of *C. burnetii*

Primer	Sequence	Size (bp)	Ref.
Com1-F	5'GAAGCGCAACAAGAAGAACAC3'	438 bp	Zhang *et al*. (1998)
Com1-R	5'TTGGAAGTTATCACGCAGTTG3'		
Trans-1F	5'TATGTATCCACCGTAGCCAGTC3'	687 bp	Dhaka *et al.*(2019)
Trans-2R	5'CCCAACAACACCTCCTTATTC3'		

Table 2. Thermal Cycle conditions used for PCR

Steps	Conditions for *IS1111* gene		Conditions for *Com1* gene	
Initial denaturation	95°C -5min		95°C-2min	
Denaturation	94°C -30s	X 35cycles	94°C-30s	X 40 cycles
Annealing	52°C-30s		63°C-4s	
Extension	72°C -1min		72°C -1min	
Final extension	72°C -10min		72°C-10min	

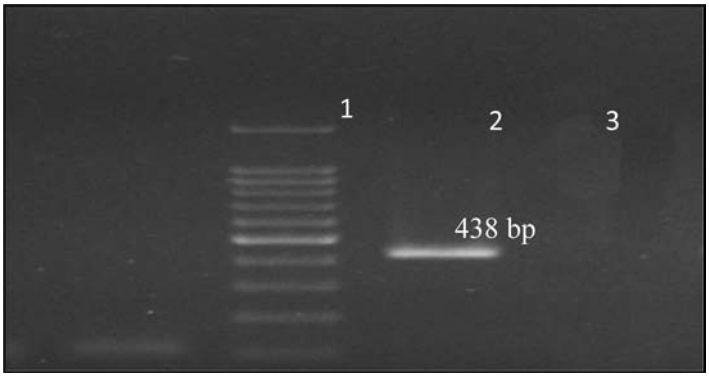

Fig. 1 PCR amplicon of *Com1* gene

Lane 1-100 bp ladder Lane 2- *Com1* Lane 3-Negative control

Fig. 2 PCR amplicon of *IS1111* gene

Lane 1-100 bp ladder Lane 2- *IS1111* Lane 3-Negative control

stating that it would be due to the single-copy nature of the *Com1* gene, which might have led to non-detection. Sameena *et al*. (2020) reported the occurrence of *C. burnetii* in goat milk by PCR in 47.37 per cent samples, which is contradicting with findings of the present study.

The targeted *IS1111* gene could not be detected in any of the milk and vaginal swab samples of goats. This finding in the present study was supported by Roest *et al*. (2013) stating that the pathogen's rate of shedding was higher in vaginal mucus compared to milk. On the contrary, Dhaka *et al.* (2017) reported a prevalence of 0.75 per cent in cattle from Kerala.

The serum was separated from clots of blood and analysed by ELISA using a commercial ELISA kit (Bio X Diagnostics, Rochefort, Belgium) for antibody detection. Out of 150 sera samples tested, 12 samples (7.99 per cent) have shown the presence of antibodies against *C. burnetii.* Of 12 seropositive samples, six, four and two were from Malappuram, Palakkad and Thrissur districts, respectively. The occurrence of antibodies against *C. burnetti* between districts was statistically non-significant (p>0.05) with a chi-square value of 3.43. Of the 150 sampled goats, 65 goats belonged to the one to two years age group, 54 goats were two to three years of age and 31 goats were above three years of age.

In the current study, the occurrence of antibodies against *C. burnetii* was higher in goats above three years of age (19.35 per cent), compared to goats of two to three years (7.40 per cent) and one to two years (3.70 per cent). The variability of the occurrence of antibodies against *C. burnetii* between the age groups of goats was statistically significant (p<0.0.5) with a Chi-square value of 6.788. The findings in the present study agreed with that of Sameena *et al*. (2020), who reported that the occurrence of *C. burnetii* antibodies was 6.31, 19.7 and 23.78 per cent in goats belonged to the age group of one to two years, two to three years and above three years, respectively. Our results varied with Kennerman *et al.* (2010) who mentioned that a higher rate of antibodies against *C. burnetii* occurred in primiparous ewes (one-year-old) compared to biparous ewes (two years old).

The study highlights the presence of antibodies against *C. burnetii* among goats with reproductive disorders in Central and Northern Kerala. Sero-detection of the pathogen poses a public threat because of its resistance to chemicals, high temperatures and disinfectants and invariable shedding in various secretions (milk) and excretions (urine, placenta and faeces).

Summary

Coxiella burnetii could not be detected in any of the collected milk (n=150) and vaginal swab samples (n=150) using PCR targeting *Com1* and *IS1111* genes. Serological diagnosis of goat blood samples (n=150) using a commercial ELISA kit revealed the occurrence (7.99 per cent) of antibodies against *C. burnetii*.

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