Int.J.Curr.Microbiol.App.Sci (2019) 8(2): 2102-2107

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 8 Number 02 (2019)
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.802.243

A Cross-sectional Study on the Occurrence of *Coxiella burnetii* Infection in a Dairy Farm, Bareilly, India

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Keywords

Coxiella burnetii, Q fever, Cattle, Dairy farm, PCR

Article Info

Accepted:
15 January 2019
Available Online:
10 February 2019

A B S T R A C T

Q fever is highly infectious bacterial zoonoses caused by *Coxiella burnetii* and remains largely neglected and underreported in various states of India. The present cross-sectional study employing a simple random sampling approach analysed a total of 324 samples (108 blood, 108 sera and 108 vaginal swabs) from cattle (n=108) employing of PCR and ELISA of cattle dairy farm from Bareilly, Uttar Pradesh, India. Besides, 18 environmental samples (animal feed-05, soil-04, drainage water-05 and drinking water-04) from the premises of the farm were also collected. On screening of cattle samples by trans-PCR and com1-PCR revealed positivity for *C. burnetii* DNA in 9.25% (10/108) and 5.55% (6/108) samples of cattle blood; 12.03% (13/108) and 5.55% (6/108) of sera, and 12.96% (14/108) and 06.48% (7/108) of vaginal swabs, respectively. Screening of cattle on the farm by commercial i-ELISA kit revealed antibodies against *C. burnetii* in the serum samples of 14.81% (16/108) cattle population.

Introduction

Q fever is a highly infectious disease of great public health importance caused by obligate intracellular, Gram negative bacterium *Coxiella burnetii*, which can successfully infect hosts ranging from mammals including domestic animals, humans and wildlife as well as reptiles, fish, birds, ticks and arthropods (Angelakis and Raoult, 2010; Cutler *et al.,* 2010; Vanderburg *et al.,* 2014; Eldin *et al.,* 2017).

The *C. burnetii* infections in animals generally known as 'Coxiellosis' are widespread in domestic ruminants, which serve as the major reservoirs of the pathogen. The disease in ruminants is frequently sub-clinical, but late abortions, stillbirths and reproductive disorders can occasionally be observed (Maurin and Raoult, 1999; Arricau-Bouvery and Rodolakis, 2005). The potential risk arising from cattle has found greater than that from small ruminants, as cattle not only excreted more number of pathogens but their

shedding in milk also lasted for a longer period (Rodolakis *et al.,* 2007).

Due to the limited diagnostic capacities, epidemiological studies on Q fever in India in general are very few (Vaidya *et al.,* 2010; Malik *et al.,* 2013; Stephen *et al.,* 2014; Kumar *et al.,* 2017; Mohan *et al.,* 2017; Dhaka *et al.,* 2017, Sahu *et al.,* 2018). It is in this context that the present study was envisaged to assess the occurrence of Q fever in a dairy farm, Bareilly, Uttar Pradesh, India.

Materials and Methods

A cross-sectional study with simple random sampling was conducted in a dairy farm of Bareilly district, Uttar Pradesh, India. A total of 324 samples (108 blood, 108 sera and 108 vaginal swabs) were collected from 108 cattle for screening of *C. burnetii* infection in the dairy herd. Additionally, 18 environmental samples (animal feed-05, soil-04, drainage water-05 and drinking water-04) from the premises of the farm were also collected for detection of *C. burnetii*. All the samples were aseptically collected in sterile containers and were transported to laboratory under chilled conditions. Blood, vaginal swabs and environmental samples were stored at refrigeration temperature, while, serum samples were stored at -20°C until further use.

Genomic DNA was isolated and purified from the blood samples of both cattle and human using Qiagen blood and tissue kit (Qiagen, Germany) as per the manufacturer's instructions. The processing of vaginal swab samples for screening by PCR assay was carried out as described by Berri *et al.,* (2000), according to which a simple boiling method was sufficient for DNA extraction from the vaginal swabs for analyzing it by PCR assay. It is noteworthy here that the boiling inactivates *C. burnetii* in sample, minimizes the risk to the laboratory personnel, and also remains an inexpensive procedure compared to other methods (Berri *et al.,* 2000). In brief, a sample of genital swab was vigorously shaken in 1 ml of PBS solution. The solution (200 µl) was then boiled for 10 min and centrifuged at 13,000 x g for 5 min and then collected supernatant was used for the PCR assays. The environmental and feed samples were processed as per the method described by Fitzpatrick *et al.,* (2010). In brief, 5 g of samples were mixed with 10 to 30 ml of Phosphate buffer saline (PBS) solution to create homogenized slurry, which was kept for 1 h at room temperature and then centrifuged for 5 min at 123 xg. The supernatant was removed and centrifuged at 20,000 xg for 15 min. The supernatant was then carefully discarded and the pellet was re-suspended in 1 ml of PBS solution. Finally, 700 µl of the re-suspended pellet was processed for DNA extraction using Qiagen Stool kit (Qiagen, USA). Following DNA extraction, purity of extracted DNA was checked using a Biospectrometer (Eppendorf GmbH, Germany). DNA with an absorption ratio (A_{260}/A_{280}) of more than or equal to 1.80 were desirable for PCR assay. The DNA of standard *C. burnetii* Nine Mile strain was thankfully received from Dr. Eric Ghigo, URMITE-IRD, Faculté de Médecine, France.

The detection of pathogen in all the collected samples was carried out by PCR employing *trans* and *com*1 genes. The trans-PCR assay was performed targeting transposons-like regions in chromosomal DNA using, trans-1 (5'-TAT GTA TCC ACC GTA GCC AGT C-3') and trans-2 (5'-CCC AAC AAC ACC TCC TTA TTC-3') with an expected amplicon size of 687 bp (Berri *et al.,* 2000) while, com1-PCR was performed using primers com 1-F (5'-AGTAGAAGCATCCCAAGC ATTG-3') and com 1-R (5'TGCCTGCTAGCTGT

AACGATTG -3') with an expected amplicon size of 501 bp (Zhang *et al.,* 1998). The cycling conditions for trans 1 and 2 primers were standardised at 94°C for 5 min (initial denaturation), followed by 40 cycles of 94°C for 30 s (denaturation), 52°C for 1 min (annealing), 72°C for 1 min (extension), and an final extension 72°C for 10 min. The cycling conditions for *com1* included an initial denaturation of DNA at 95°C for 5 min followed by 30 cycles, each consisting of denaturation at 95°C for 30s, annealing at 63°C for 1 min and extension at 72°C for 1 min. A final extension was provided at 72°C for 10 min followed by holding the tubes at 4°C. The DNA of *C. burnetii* Nine Mile 1 strain was used as a positive control whereas, Nuclease free water (Thermoscientific, USA) served as negative template control. The resultant PCR products were visualized after electrophoresis using gel documentation system (UVP Gel Seq Software). The sera samples obtained from cattle were screened using commercial i-ELISA kits (Bio-X Diagnostics, Rochefort, Belgique) as per manufacturer's instructions.

Results and Discussion

The results of 324 clinical samples (blood-108, serum-108 and vaginal swabs-108) collected from cattle (n=108), as well as environmental samples (n=18) from the dairy farm, screened for coxiellosis are summarized in tabular form (Table 1). The trans-PCR assay targeting *IS1111* gene of *C. burnetii* in the DNA of cattle (n=108) showed the presence of pathogen in 12.03% (13/108) blood samples, 09.25% sera (10/108) and 12.96% (14/108) vaginal swabs, respectively (Table 1); while the com1-PCR assay targeting *com1,* the single copy gene of *C. burnetii,* could detect the pathogen in lesser number of samples, with a positivity recorded as 05.55% (6/108) in blood samples, 05.55% (6/108) sera and 06.48% (7/108) vaginal swabs (Table 1). All the samples collected from environment (n=18) of the farm tested negative in both the PCR assays (Table 1). The commercial ELISA kit revealed seropositivity for coxiellosis in 14.81% (16/108) of cattle on the dairy farm.

In the present study, a higher detection rate of pathogen in cattle blood samples by trans-PCR assay observed to the tune of 12.03% (13/108), and 12.96% (14/108) in blood/sera samples and vaginal swabs, respectively; as compared to 05.55% (6/108), and 06.48% (7/108) by com1-PCR assay, respectively. It can be attributed to the reported higher sensitivity of the former assay targeting *IS1111* gene, a multi-copy gene having 7-110 copies per isolate of *C. burnetii* (Klee *et al,* 2006), as compared to the latter test targeting *com1* gene, reported as a single-copy gene (Kersh *et al.,* 2012). These observations corroborates with some earlier reported findings about the higher sensitivity of trans-PCR than com1-PCR, wherein the pathogen detection in clinical samples by these tests has been reported as 59% and 44% (Turra *et al.,* 2006), 63% and 30% (Kersh *et al.,* 2012), 64% and 10% (Shapiro *et al.,* 2016), respectively.

The negativity of all the environmental samples collected from premises of all the three gaushalas in PCR assays observed in our study might be either due to the absence of the organism in these samples, or non-shedding of the pathogen in vaginal mucous in recent past, or any possible PCR inhibition in the environmental samples, as has been experienced in some earlier studies related to the screening of environmental samples (Abolmaaty *et al.,* 2007; de Bruin *et al.,* 2011).

The detection of antibodies against *C. burnetii* by commercial ELISA kit in 14.81% (16/108) of serum samples of cattle is in accordance

with earlier studies, wherein the prevalence of bovine coxiellosis has been reported to range from 5.55% to 29.9% (Kaplan and Bertagna, 1955; Joshi *et al.,* 1978; Vaidya *et al.,* 2010; Malik *et al.,* 2013; Das *et al.,* 2014), however, it was lower than the median prevalence of *C. burnetii* infection among cattle reported as 19.4% at the animal level and 37.7% at the herd level (Guatteo *et al.,* 2011).

Table.1 Results of sample screening by PCR assays and ELISA for coxiellosis at Cattle Dairy farm (Bareilly, Uttar Pradesh)

Category	Types of samples	Samples screened	Positivity for coxiellosis by different tests		
			PCR assays		ELISA % (positive)
			Trans-PCR % (positive)	Com 1-PCR % (positive)	
Cattle (108)	Blood	108	09.25% (10/108)	05.55% (6/108)	NA
	Serum	108	12.03% (13/108)	05.55% (6/108)	14.81% (16/108)
	Vaginal swabs	108	12.96% (14/108)	06.48% (7/108)	NA
Environmental samples (18)	Feed	5	-	-	NA
	Soil	4	-	-	NA
	Water	4	-	-	NA
	Sewage	5	-	-	NA

In conclusion, screening of dairy Farm by trans-PCR and com1-PCR revealed positivity for *C. burnetii* DNA in 9.25% (10/108) and 5.55% (6/108) samples of cattle blood; 12.03% (13/108) and 5.55% (6/108) of sera, and 12.96% (14/108) and 06.48% (7/108) of vaginal swabs, respectively. Screening of cattle on the farm by commercial i-ELISA kit revealed antibodies against *C. burnetii* in the serum samples of 14.81% (16/108) cattle population. We further propose to undertake more number of epidemiological investigations particularly in farms to identify possible risk factors that facilitate the transmission of this agent.

Acknowledgement

The authors thank Director, ICAR- Indian Veterinary Research Institute, Izatnagar for providing facilities to carry out the research. The research is supported by grants from the Indian Council of Agricultural Research-Outreach Programme on Zoonotic Diseases (Grant no.1000494) to SVS-M. The technical assistance by Mr. K.K. Bhat and Dr. Deepa Ujjawal is duly acknowledged.

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How to cite this article:

Manesh Kumar, Satyaveer Singh Malik, Sunitha Ramanjeneya, Radhakrishna Sahu, Jess Vergis, Richa Pathak, Pankaj Dhaka, Jay Prakash Yadav, Sukhadeo Baliram Barbuddhe and Deepak Bhiwa Rawool. 2019. A cross-sectional Study on the Occurrence of *Coxiella burnetii* Infection in a Dairy Farm, Bareilly, India. *Int.J.Curr.Microbiol.App.Sci.* 8(02): 2102-2107.
doi: https://doi.org/10.20546/ijcmas.2019.802.243