www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; SP-9(4): 39-41

www.thepharmajournal.com
Received: 04-02-2020
Accepted: 06-03-2020

Sameena
Veterinary Public health and Epidemiology P.V. Narsimha Rao Telangana University, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

A Vijaya Kumar
Veterinary Public health and Epidemiology P.V. Narsimha Rao Telangana University, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Sujatha Sing
Veterinary Public health and Epidemiology P.V. Narsimha Rao Telangana University, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

N Krishnaiah
Veterinary Public health and Epidemiology P.V. Narsimha Rao Telangana University, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

A Poojasree
Veterinary Public health and Epidemiology P.V. Narsimha Rao Telangana University, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
Sameena
Veterinary Public health and Epidemiology P.V. Narsimha Rao Telangana University, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana, India

Sero-Occurrence and molecular detection of *Coxiella burnetii* in sheep and goat milk of Telangana state

Sameena, A Vijaya Kumar, Sujatha Sing, N Krishnaiah and A Poojasree

Abstract
The aim of this study was to estimate the seroprevalence and molecular detection of *Coxiella burnetii* in sheep and goat milk of Telangana state. A total of 36 pooled milk samples (17 sheep and 19 goat) were collected from different areas of Telangana state. The milk samples were tested by enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction with specific primers trans and com 1 gene. In the present study, out of 36 pooled milk samples 12 (33.33%) and 16 (44.44%) were positive by ELISA and PCR respectively. Out of 17 sheep and 19 goat milk samples 5 (29.41%), 7 (36.48%) were tested antibodies positive against *Coxiella burnetii* using commercial ELISA kit and 7 (41.18%), 9 (47.37%) by PCR respectively.

Keywords: Sero-Occurrence, *Coxiella burnetii*, sheep and goat milk

Introduction
Q fever is a zoonotic disease caused by *Coxiella burnetii*, a small obligate intracellular Gram-negative pathogen worldwide spread, except New Zealand (Maurin and Raoult, 1999 [9]; YinMing-Yang *et al*., 2015) [18]. Sheep and goats are primary reservoir for man. In a recent work, sheep have been shown to eliminate *Coxiella burnetii* mostly through vaginal mucous or feces, while goat mainly through milk (Rodolakis, 2009) [12]. Goats get this infection by inhalation of aerosols or by uptake from the environment (Roest *et al*., 2012) [13] and the infection is generally considered as asymptomatic. The most common clinical signs of chronic Q fever are abortions and stillbirths. In sheep and goat flocks with reproductive disorders, animals contemporarily shed the bacterium through vaginal mucus, feces and milk. *Coxiella burnetii* was a cause of abortion waves at 2 dairy sheep farms and 28 dairy goat farms in the Netherlands. It persists for several years and is probably lifelong.
Placenta, amniotic fluid, fetal membranes and lochia of an infected sheep or goat contain millions of bacteria following abortion or normal delivery (Rousset *et al*., 2007; Wouda and Dercksen, 2007; Roest *et al*., 2012) [14, 16, 13]. *Coxiella burnetii* has been considered as a potential weapon for bioterrorism and classified as a Category 'B' critical biological agent by the Centre for Diseases Control and Prevention (Kirkan *et al*., 2008) [5]. It is placed among the 13 global priority zoonoses. As per International Livestock Research Institute (ILRI), it is considered as 'the most contagious disease' as infectious dose of the agent can be as low as one organism (ILRI, 2012) [3].
Coxiella burnetii is secreted in the milk, therefore the ingestion of contaminated food such as raw milk and dairy products were source of infection for humans (Maurin and Raoult, 1999) [9]. Much work was not carried out in India on *Coxiella burnetii* in sheep and goat milk, therefore an attempt was made to study on occurrence of *Coxiella burnetii* in small ruminants of Telangana State.

Materials and Methods
Sample collection: A total of 36 blood samples (17 sheep and 19 goat) were collected from different areas of Telangana state. Milk samples were transferred in sterile screw capped tubes and stored at -20^{0}C, until processed, Department of veterinary public health and epidemiology, College of CVSC Rajendranagar, Hyderabad.

Sero occurrence of sheep Coxiellosis by ELISA
A Commercial ELISA kit (Bio X diagnostics) was used for detection of antibodies against *Coxiella burnetii* in milk of sheep and goat samples.

DNA isolation and PCR

Two primers, targeting trans and com1 genes were used for detection of *Coxiella burnetii* DNA in milk samples collected from sheep and goat. Primer sequences and their product size are indicated below.

Primer Sequence	Product Size (bp)	Reference
trans-1 (5'-TAT GTA TCC ACC GTA GCC AGT C-3')	687 bp	Lorenz *et al*., 1998 [7]
trans-2 (5'-CCC AAC AAC ACC TCC TTA TTC-3')	687 bp	Lorenz *et al*., 1998 [7]
com-1F (5'-GAA GCG CAA CAA GAA GAA CAC-3')	438bp	Zhang *et al*., 1998 [19]
com-1R (5'-TTG GAA GTT ATC ACG CAG TTG-3')	438bp	Zhang *et al*., 1998 [19]

DNA purification from milk

The method followed by Mohammed *et al*. (2014) [10] was used in present study. One ml of milk was pipetted into 1.5 ml micropipette tube and centrifuged at 13000x g for 60 min. Pellet was separated from cream and milk layers. 180 µl ATL buffer and 20 µl proteinase K were added to the pellet followed by mixing thoroughly by vortexing for 15 seconds. Then it was kept overnight at room temperature. Further steps followed in this protocol were identical to that of performed in case of DNA extraction from blood.

Screening of samples

Polymerase chain reaction was performed to detect *Coxiella burnetii* DNA in milk samples. Two genes were targeted namely trans and com1.

PCR amplification of the trans 1 and com 1 gene fragment was set up to 25 µl reactions. The PCR protocol was initially standardized by optimizing the concentration of the components of the reaction mixture in the PCR assay and by varying the annealing temperature and cycling conditions. (Table. 3.). A 25 µl reaction mixture was set up for each sample, comprising of a 12.5 µl 2X Master Mix, 0.5-1 U Taq DNA Polymerase, 1 µl of each primer (com1 forward and reverse), and 2 µl DNA. Finally, the volume was adjusted to 25 µl using molecular grade water. Purified DNA of *Coxiella burnetii* as positive control was provided by Centre for Zoonosis, Dept of Veterinary Public Health and Epidemiology, Nagpur Veterinary College, Maharashtra. Cycling conditions used for two sets of coxiella primer given in table: 1. The amplified PCR products along with 100bp ladder were resolved in 1% agarose gel containing ethidium bromide.

Table 1: Cycling conditions used for two sets of coxiella primer

S. No	Step	*Trans*	*Com 1*
1.	Initial denaturation	94 ºC /5min	95 ºC /2min
2.	Final denaturation	94 ºC/30 sec	94 ºC/30 sec
3.	Annealing	52 ºC/1min	63 ºC/45 sec
4.	Initial extension	72 ºC/1 min	72 ºC/1 min
5.	Final extension	72 ºC/ 10 min	72 ºC/ 10 min
6.	Hold	4 ºC	4 ºC

Results and Discussion

Occurrence of *Coxiella burnetii* in milk samples are shown in table 2**.** Out of 36 milk samples (17 from sheep, 19 from goat) 16 (44.44%) were positive by PCR compared to 12 (33.33%) by ELISA and the percent of ELISA compared to PCR was 75.00%, of which 17 sheep milk samples gave 7 (41.18%) and 5 (29.41%) were positive by PCR and ELISA respectively with 71.43% efficiency of ELISA compared to PCR. Out of 19 goat milk samples 7 (36.84%) and 9 (47.37%) were positive by ELISA and PCR respectively with 77.78% efficiency of ELISA compared to PCR.

The prevalence of *Coxiella burnetii* in sheep milk by ELISA was 29.41% in present study, which was higher than the prevalence (17%) by using IFA reported by Khalifa *et al*. (2016) [4], whereas no prevalence in milk samples reported by Dogru *et al*. (2010) [1].

The prevalence of *Coxiella burnetii* in sheep milk by PCR was 41.18% in present study, which was higher than the prevalence of 22%, 10.71%, 4%, 3.5% and 1.42% reported by Garcia-perez *et al*. (2009) [2], Vaidya *et al*. (2010) [15], Yesim can *et al*. (2015) [17], Ongor *et al*. (2004) [11] and Malik *et al*. (2013) [8] respectively.

The prevalence of *Coxiella burnetii* in goat milk by ELISA was 36.84% in present study, which was higher than the prevalence of 19% using by IFA reported by Khalifa *et al*. (2016) [4]. The prevalence of *Coxiella burnetii* in goat milk by PCR was 47.37% in present study, which was higher than the prevalence of 4% and 2.8% reported by Yesim Can *et al*. (2015) [17] and Vaidya *et al*. (2010) [15] respectively.

Table 2: Occurrence of *Coxiella burnetii* in milk samples

S. no	Samples	No. of Samples	ELISA positive		PCR positive		Percent of serological compared to PCR %
			No.	%	No.	%	
1	Sheep milk	17	5	29.41	7	41.18	71.43
2	Goat milk	19	7	36.84	9	47.37	77.78
Total milk		36	12	33.33	16	44.44	75.00

The prevalence of *Coxiella burnetii* in pooled milk (sheep and goat) by ELISA was 33.33% in present study, which was higher than the prevalence of 18% using IFA reported by Khalifa *et al*. (2016) [4]. The prevalence of *Coxiella burnetii* in pooled milk by PCR was 44.44% in present study, was higher than the prevalence of 6.25%, 6%, 4.63%and 3% reported by Vaidya *et al*. (2010) [15], Yesim Can *et al*. (2015) [17], Malik *et al*. (2013) [8] respectively.

Conclusion

The prevalence of Coxiella burnetii in sheep and goat in Telangana state was 33.33%, which is quite alarming situation, necessary steps should be taken for prevention and control of this infection. The extreme persistence of *C. burnetii* in the environment and its diffusion in domestic ruminants and wild animals indicated therefore that good management practices played an important critical role for the control of Q fever, not only in animals but also in man.

References

1. Dogru AK, Yildirim M, Unal N, Gazyagci S. The relationship of *Coxiella burnetii*seropositivity between farm animals and their owners: A pilot study, Journal of Animal and Veterinary Advances. 2010; 9:1625-1629. doi: dx.doi.org/10.3923/javaa.2010.1625.1629.
2. Garcia-Perez AI, Astobiza I, Barandika JF, Atxaerandio R, Hurtado A, andJuste RA. Investigation of *Coxiella burnetii* occurrence in dairy sheep flocks by bulk tankmilk analysis and antibody levels determination. Journal of Dairy Science. 2009; 92:1581-1584.

3. ILRI. International Livestock Research Institute. Mapping of poverty and likelyzoonoses hotspots. Report to department for international development, UK, 2012.
4. Khalifa NO, Elhofy FI, Fahmy HA, Sobhy MM, Agag MA. Seropervelance and molecular detection of *Coxiella burnetii* infection in sheep, goats and human in Egypt. ISOI Journal of Microbiology, Biotechnology and Food Science. 2016; 2:1-7.
5. Kirkan S, Kaya O, Tekbiyik S, Parin U. Detection of *Coxiella burnetii* in cattle by PCR. Turkish Journal of Veterinary and Animal Sciences. 2008; 32(3):215-220.
6. Klaasen M, Roest H, Van der Hoek W, Goossens B, Secka A, Stegeman A. *Coxiella burnetii* seroprevalence in small ruminants in Gambia. Plos One. 2014; 9(1):e85424.
7. Lorenz H, Jager C, Willems H, Balger G. PCR detection of Coxiella burnetii from different clinical specimens, especially bovine milk on the basis of DNA preparation with silica matrix. Applied Environment and. Microbiology. 1998; 64:4234-4237.
8. Malik SS, Das DP, Rawool D, Kumar A, Suryawanshi RD, Negi M *et al*. Screening of foods of animal origin for *Coxiella burnetii* in India. Epidemiology, 2013, 08-27.
9. Maurin M, Raoult D. Q fever. Clinical Microbiology Reviews. 1999; 12:518-553.
10. Mohammed Osama B, Abdulrahman Jarelnabi A, Riyadh S, Aljumaah Mohammed A, Alshaikh Amel O, Bakhiet Sawsan A *et al*. Coxiella burnetii, the causative agent of Q fever in Saudi Arabia: molecular detection from camel and other domestic livestock. Asian Pacific Journal of Tropical Medicine. 2014; 7(9):715-719.
11. Ongor H, Cetinkaya B, Karahan M, Acik MN, Bulut H, Muz A. Detection of *Coxiella burnetii* by immunomagnetic separation-PCR in the milk of sheep in Turkey. Veterinary Record. 2004; 154:570-572. doi: dx.doi.org/10.1136/vr.154.18.570.
12. Rodolakis A. Q fever in dairy animals. Annals of the New York Academy of Sciences. 2009; 1166:90-93.
13. Roest HJ, Van Gelderen B, Dinkla A, Frangoulidis D, Van Zijderveld F, Rebel A *et al*. Q fever in pregnant goats: pathogenesis and excretion of Coxiella burnetii. Plos One, 2012, 7e48949.
14. Rousset E, Durand B, Berri M, Dufour P, Prigent M, Russo P *et al*. Comparative diagnostic potential of three serologicaltests for abortive Q fever in goat herds. Veterinary Microbiology. 2007; 124:286-297.
15. Vaidya VM, Malik SVS, Bhilegaonkar KN, Rathore RS, Kaur S, Barbuddhe SB. Prevalence of Q fever in domestic animals with reproductive disorders. Comparative immunology, microbiology and infectious diseases. 2010; 33(4):307-321.
16. Wouda W, Dercksen DP. Abortion and stillbirth among dairy goats as a consequenceof *Coxiella burnetii*. *Tijdschr*. Diergeneeskd. 2007; 132(23):908- 911.
17. Yesim Can H, Elmali M, Karagoz A. Detection of *Coxiella burnetii* in cows', goats', and ewes' bulk milk samples using polymerase chain reaction (PCR). *Mljekarstvo/Dairy*. 2015; 65(1).
18. Yin Ming-Yang, Qin Si-Xian, Tan Qi-Dong, Feng Sheng- Yong, Liu Guang-Xue, Zhbu Dong- Hui *et al*. burnetti seroprevelance in Tibetan sheep in China. Vector-Borne and Zoonotic Diseases. 2015; 15(7):419-422.
19. Zhang, GQ, Nguyen SV, To H, Ogawa M, Hotta A, Yamaguchi T *et al*. Clinical evaluation of a new PCR assay for detection of Coxiella burnetii in human serum samples. Journal of Clinical Microbiology. 1998; 36(1):77-80.