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Detection of *Coxiella burnetii* in Aborted Fetuses of Cattle and Sheep Using Polymerase Chain Reaction Assay in Mashhad City, Iran

Zeinab Abiri,[1,*] Mohammad Khalili,[1] Mehrnaz Rad,[2,3] and Hamid Sharifi[4,5]

[1]Department of Pathobiology, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, IR Iran
[2]Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, IR Iran
[3]Center of Excellence in Ruminant Abortion and Neonatal Mortality, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, IR Iran
[4]Research Center for Modeling in Health, Institute for Futures Studies in Health, Kerman University of Medical Sciences, Kerman, IR Iran
[5]Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, IR Iran

*Corresponding author: Zeinab Abiri, Department of Pathobiology, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, IR Iran. Tel: +98-9159350178, Fax: 03-33222047, E-mail: abiri@vet.uk.ac.ir

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Abstract

Background: *Coxiella burnetii* is an important intracellular pathogen that ruminants can act as primary reservoirs. Reservoirs may excrete the bacterium into the placenta, vaginal mucus and feces.
Objectives: The aim of this study was to detect *C. burnetii* in aborted samples from ruminant flocks in Mashhad city, northeast of Iran, using the polymerase chain reaction (PCR) assay.
Materials and Methods: A total number of 154 fetal tissue samples of cattle, sheep and goat were subjected to nested PCR assay.
Results: Sixteen (17.3%) out of 92 samples from sheep and 15 (25%) from 60 cattle fetuses were positive.
Conclusions: The results of this study indicate the presence of *C. burnetii* in aborted ruminants and these can be the potential reservoirs of *C. burnetii* in the mentioned area.

Keywords: Fetus, Ruminants, PCR, *Coxiella burnetii*

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1. Background

Coxiella burnetii is an important intracellular pathogen that has been implicated in cases of Q fever, a zoonotic worldwide disease with acute and chronic stages. Ruminants (cattle, sheep and goats) can act as primary reservoirs of *C. burnetii* and a variety of species like humans, small rodents, dogs, cats, birds, fish, reptiles and arthropods may be infected (1, 2).

There is a list of symptoms commonly seen with acute Q fever in human that the combination of them varies from person to person; high fevers (up to 40°C - 40.5°C), severe headache, general malaise, myalgia, chills and/or sweats, nonproductive cough, nausea, vomiting, diarrhea, abdominal and chest pain are the most important signs. The most common clinical signs in animals are pneumonia, abortion, still birth and delivery of weak offspring (3) that can lead to economic losses.

Reservoirs may excrete the bacterium into the placenta, vaginal mucus and feces (4). *C. burnetii* have a stable small-cell variant (SCV) form (5) that is extremely sustainable and virulent (6); *C. burnetii* may survive in environmental conditions and foods (fresh meat, dry milk powder etc.) for several months (7, 8). Veterinary personnel, farmers (also their employees and families), stockyard workers, shearers, animal transporters, laboratory and abattoirs workers are occupations that are at risk of infection (9).

Diagnosis of the pathogen is usually based on serological methods such as complement fixation (CF) test which is prescribed by office international des epizooties (OIE) as a diagnostic method for *C. burnetii* and also enzyme-linked immunosorbent assay (ELISA). In aborted ruminants detection of *C. burnetii* has been done using staining techniques, such as Stamp, Gimenez, and Machiavelli, followed by a serological analysis (10). Staining techniques cannot be specific and the CF test has low sensitivity (11). Antibodies of *C. burnetii* in sheep and goats cannot be detected frequently by specific antigen (10). Polymerase chain reaction (PCR) is increasingly used as a sensitive and specific method (12, 13) for detection of the bacteria in samples using primers based on a transposon-like repetitive region (14).

Various infectious and noninfectious reasons are reported in relation to incidence of ovine abortion (15, 16). Masala et al. (2004) reported the presence of the *C. burnetii* in 10% of analyzed fetuses in Italy by PCR (17). In Iran, there are very limited studies on presence of *C. burnetii* in flocks with abortion (18).

2. Objectives

The aim of this study was to detect the *C. burnetii* in

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aborted samples from sheep and goat flocks in Mashhad city, Iran, using PCR.

3. Materials and Methods

3.1. Sample Preparation and DNA Extraction

A total of 154 fetal tissue samples (92 ovine, 2 caprine and 60 bovine) were obtained from center of excellence in ruminant abortion and neonatal mortality, school of veterinary medicine, Ferdowsi university of Mashhad.

For avoiding cross contamination during sampling, farmers referred the complete aborted fetuses to the center immediately, and then samples were taken from deep tissues and an internal organ (liver) under sterile conditions.

DNA was manually extracted from the tissues using phenol-chloroform method; briefly, 10 - 20 mg fetal tissue was dissected from each sample using a razor blade/scalpel. Minced tissues were digested in 600 μL cell lysis buffer (50 mM Tris-HCl, 1 mM ethylene diamine tetraacetic acid (EDTA) 0.1% (w/v) tween 20) and 10 μL proteinase K and the solution was incubated at 37°C overnight. Then an equal volume of phenol chloroform isoamyl alcohol (PCI; 25:24:1 ratio) was added to each digested tissue and the suspension was mixed gently for 5 minutes by rocking platform and was centrifuged for 10 minute at 10000 rpm at room temperature. Then top (aqueous) phase containing the DNA was transferred to a new tube and was mixed with equal volume of chloroform-isoamyl alcohol (24:1).

After gently shaking for 2 minutes and centrifuging for 1 minute at 10,000 rpm, 2.5μL ice-cold pure ethanol was added and the solution was placed at −70°C. After 1 hour, the tubes were centrifuged for 20 minutes at maximum speed in a fixed-angle microcentrifuge. Supernatant was removed and 1 mL of room-temperature ethanol 70% was added to pellets. Then, the tubes were left at room temperature to achieve dry pellets. Finally, pellets were resuspended in 100 μL sterile distilled water and stored at −20°C until use as DNA template.

3.2. Polymerase Chain Reaction Assay

For detection of *IS1111* gene, nested PCR assay was conducted using the specific primers as previously described (Table 1) (19, 20). These primers were designed based on a repetitive, transposon-like element with the high specificity and sensitivity for the molecular diagnosis of *C. burnetii* (21).

Table 1. The Sequences of the Primers Used in This Study

Protocol	Sequence	Gene	Size, bp
Trans PCR		*IS1111*	687
Trans1	TATGTATCCACCGTAGCCAGT		
Trans2	CCCAACAACACCTCCTTATTC		
Nested PCR		*IS1111*	203
261 F	GAGCGAACCATTGGTATCG		
463 R	CTTTAACAGCGCTTGAACGT		

Abbreviation: PCR, polymerase chain reaction.

Nested PCR was performed by two amplifications. At the first one, PCR was done in a total volume of 25 μL containing 5 μL DNA template, 2 U Taq DNA polymerase (Bioneer, Korea), 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 3 mM MgCl2, 1 mM deoxynucleoside triphosphate mix 10 mM of each primer and water up to volume of reaction. The thermal cycles for first PCR contains: an initial denaturation of DNA at 95°C for 3 minutes, followed by five cycles at 94°C for 30 seconds, 66 to 61°C (the temperature was decreased by 1°C between consecutive steps) for 1 minute, and 72°C for 1 minute. These cycles were followed by 35 cycles consisting of 94°C for 30 seconds, 61°C for 30 seconds, and 72°C for 1 minute and then a final extension step of 10 minutes at 72°C. Second PCR was performed with 261 F and 463 R primers and the cycling conditions included an initial denaturation of DNA at 95 °C for 3 minutes followed by 35 cycles consisting of 94°C for 30 seconds, 50°C for 45 seconds, and 72°C for 1 minute and then a final extension step of 10 minutes at 72°C.

Positive (The DNA from the *C. burnetii* Nine Mile II, strain RSA 493) and negative controls (ultra-pure sterile water) were used in all amplifications. Amplicons were visualized on agarose gel (1.5%) electrophoresed at 90 V for 30 minutes and stained with ethidium bromide at the final concentration of 0.5mg. mL-1. Also, photos were documented in Gel Doc 1000 (Vilber Lourmat, France).

3.3. Statistical Analysis

Data were analyzed using descriptive statistics for binary data and 95% confidence interval (CI). A chi-square test was used to compare the prevalence of the presence of infection in different species. Stata 11.2 was used to analyze the data.

4. Results

A total number of 154 fetal tissue samples of cattle, sheep and goat were subjected to nested PCR assay for detection of *IS1111* gene (Figure 1).

In total, 31 (20.1; 95% CI: 14.1 - 27.3) samples harbored the tested gene; 16 (17.3%; 95% CI: 10.3 - 26.7) samples out of 92 from sheep contained the tested gene. Also, 15 (25%; 95% CI: 14.7 - 37.9) PCR-positive templates from 60 cattle fetuses were detected too. Any samples from goat were not positive in this study. There was no significant difference on the prevalence of infection in sheep and cattle samples (P = 0.25)

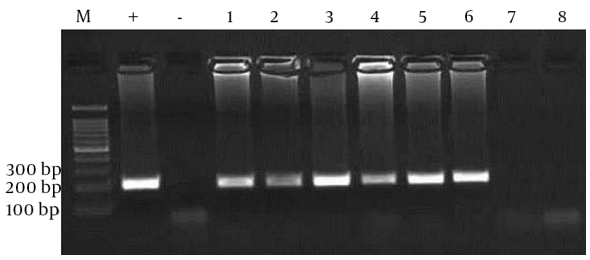

Figure 1. Lanes 1 - 6, positive samples; lane 7 - 8, negative samples; lane +, positive control (DNA template from the standard *C. burnetii* Nine Mile); lane −, negative control (ultra-pure sterile water) and lane M, 100-bp DNA ladder

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In this study, in the most of samples, abortion occurred in late pregnancy. Also, abortions in sheep mostly have been occurred in December and January. However, most of abortions in cattle were in June and July.

5. Discussion

The results of this study indicated the presence of *C. burnetii* in abortion fetal samples from sheep, goat and cattle flocks in Mashhad city, Iran. In the present study, the prevalence of *C. burnetii* was higher in cattle (25%) than in sheep (17.3%) and no positive sample found in goat. The prevalence rates of the pathogen in sheep, cattle and goat in similar study in Turkey were 11.11%, 3.92% and 40%, respectively (22). *Coxiella burnetii* was also found in 7.3% of cattle and 11.1% of goat herds of England and Wales but *C. burnetii* was not detected in any of the sheep herds (23). In another study done in northern Cyprus, 35% of bovine, 33% of sheep and 50% of goat abortion cases were positive for *C. burnetii* (24). Although abortions in goats due to coxiellosis occur more than in sheep (25), in this study there was no positive sample in goats and this can be due to the number of goat samples that was low and could not be properly assessed.

The observed variations in the results of our study and other researches may be associated to management practice, climatic conditions, sampling techniques and detection method. Staining techniques and serological methods like CFT test and ELISA has been reported as routine diagnostic assays (10). Findings indicate that PCR can be a great and useful diagnostic tool for detection of *C. burnetii* in abortion materials (26-29).

Many studies have demonstrated the role of *C. burnetti* in abortion in sheep, goat and cattle flocks (30). Examined samples in this study have already been checked for the presence of several microbial pathogens which can cause abortion or fertility reduction in ruminants; which samples were negative for Chlamydia, Toxoplasma and bovine viral diarrhea virus. Thus *C. burnetii* probably can be one of the causes of abortion in these samples.

Epidemiological researches have reported that abortion waves on dairy goat flocks could be a source for human infection especially people living near to the flocks (31). Humans are infected through inhalation of aerosols generated from contaminated placentas and body fluids (3).

Studies showed that after an abortion in ruminants, there is an important risk of direct or indirect exposure within and between herds and to the human population (32, 33). Furthermore, a study showed that the bacteria were shed by some goats for almost 4 months after an outbreak and two successive parturitions (34). Epidemiological studies suggest that goats and dairy cows represent the most important source for human because infection in these animals often is chronic (35).

Among the most important management schemes to control the transmission of *C. burnetii* in infected herds, It can be useful to provide a special location for the parturition that should be regularly disinfected and remove placenta and aborted fetuses immediately to avoid ingestion by carnivores. (3). Also, destruction of every high risk material, including contaminated bedding with incineration or burial with lime is recommended (36). Manure should be treated with lime or calcium cyanide as soon as possible. The quantities of *C. burnetii* shedding during parturition and abortions can be minimized by preventive antibiotic treatments such as tetracyclines (37).

In conclusion, this study established the presence of *C. burnetii* in aborted sheep and cattle in Mashhad city. Findings of the present study showed that sheep and cattle can be the potential reservoirs of *C. burnetii* in the mentioned area. Also, abortion materials are one of the most important transmission ways to the environment. Future researches needs to be performed on larger sample sizes from other regions for better understanding the epidemiology of *C. burnetii*.

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Footnotes

Authors' Contribution: Mohammad Khalili, and Zeinab Abiri: study concept and design and analysis and interpretation of data; Zeinab Abiri: drafting of the manuscript; Mohammad Khalili, Mehrnaz Rad, and Hamid Sharifi: critical revision of the manuscript for important intellectual content; Hamid Sharifi: statistical analysis.

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References

1. Ioannou I, Chochlakis D, Kasinis N, Anayiotos P, Lyssandrou A, Papadopoulos B, et al. Carriage of Rickettsia spp., Coxiella burnetii and Anaplasma spp. by endemic and migratory wild birds and their ectoparasites in Cyprus. *Clin Microbiol Infect.* 2009;**15 Suppl 2**:158–60. doi: 10.1111/j.1469-0691.2008.02207.x. [PubMed: 19281460]
2. Ioannou I, Sandalakis V, Kassinis N, Chochlakis D, Papadopoulos B, Loukaides F, et al. Tick-borne bacteria in mouflons and their ectoparasites in Cyprus. *J Wildl Dis.* 2011;**47**(2):300–6. doi: 10.7589/0090-3558-47.2.300. [PubMed: 21441182]
3. Arricau-Bouvery N, Rodolakis A. Is Q fever an emerging or re-emerging zoonosis? *Vet Res.* 2005;**36**(3):327–49. doi: 10.1051/vetres:2005010. [PubMed: 15845229]
4. Jones RM, Twomey DF, Hannon S, Errington J, Pritchard GC, Sawyer J. Detection of Coxiella burnetii in placenta and abortion samples from British ruminants using real-time PCR. *Vet Rec.* 2010;**167**(25):965–7. doi: 10.1136/vr.c4040. [PubMed: 21262712]
5. Coleman SA, Fischer ER, Howe D, Mead DJ, Heinzen RA. Temporal analysis of Coxiella burnetii morphological differentiation. *J Bacteriol.* 2004;**186**(21):7344–52. doi: 10.1128/JB.186.21.7344-7352.2004. [PubMed: 15489446]
6. McCaul TF, Williams JC. Developmental cycle of Coxiella burnetii:

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structure and morphogenesis of vegetative and sporogenic differentiations. *J Bacteriol.* 1981;**147**(3):1063–76. [PubMed: 7275931]
7. Bjork A, Marsden-Haug N, Nett RJ, Kersh GJ, Nicholson W, Gibson D, et al. First reported multistate human Q fever outbreak in the United States, 2011. *Vector Borne Zoonotic Dis.* 2014;**14**(2):111–7. doi: 10.1089/vbz.2012.1202. [PubMed: 24350648]
8. Gurtler L, Bauerfeind U, Blumel J, Burger R, Drosten C, Groner A, et al. Coxiella burnetii - Pathogenic Agent of Q (Query) Fever. *Transfus Med Hemother.* 2014;**41**(1):60–72. doi: 10.1159/000357107. [PubMed: 24659949]
9. Georgiou E. *Coxiella burnetii as the causative agent of abortions in sheep and goats in Cyprus*. London: Middlesex University; 2013.
10. Kovacova E, Kazar J, Spanelova D. Suitability of various Coxiella burnetii antigen preparations for detection of serum antibodies by various tests. *Acta Virol.* 1998;**42**(6):365–8. [PubMed: 10358741]
11. Berri M, Laroucau K, Rodolakis A. The detection of Coxiella burnetii from ovine genital swabs, milk and fecal samples by the use of a single touchdown polymerase chain reaction. *Vet Microbiol.* 2000;**72**(3-4):285–93. [PubMed: 10727838]
12. Kim SG, Kim EH, Lafferty CJ, Dubovi E. Coxiella burnetii in bulk tank milk samples, United States. *Emerg Infect Dis.* 2005;**11**(4):619–21. doi: 10.3201/eid1104.041036. [PubMed: 15829205]
13. 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. *Vet Rec.* 2004;**154**(18):570–2. [PubMed: 15144006]
14. Can HY, Elmalı M, Karagöz A. Detection of Coxiella burnetii in cows', goats', and ewes' bulk milk samples using polymerase chain reaction (PCR). *Mljekarstvo.* 2015;**65**(1):26–31. doi: 10.15567/mljekarstvo.2015.0104.
15. Dubey JP, Kirkbride CA. Toxoplasmosis and other causes of abortions in sheep from north central United States. *J Am Vet Med Assoc.* 1990;**196**(2):287–90. [PubMed: 2298653]
16. Kirkbride CA. Diagnoses in 1,784 ovine abortions and stillbirths. *J Vet Diagn Invest.* 1993;**5**(3):398–402. [PubMed: 8373854]
17. Masala G, Porcu R, Sanna G, Chessa G, Cillara G, Chisu V, et al. Occurrence, distribution, and role in abortion of Coxiella burnetii in sheep and goats in Sardinia, Italy. *Vet Microbiol.* 2004;**99**(3-4):301–5. doi: 10.1016/j.vetmic.2004.01.006. [PubMed: 15066733]
18. Dehkordi FS. Prevalence study of Coxiella burnetii in aborted ovine and caprine fetuses by evaluation of nested and real-time PCR assays. *Am J Anim Vet Sci.* 2011;**6**(4):180–6. doi: 10.3844/ajavsp.2011.180.186.
19. Parisi A, Fraccalvieri R, Cafiero M, Miccolupo A, Padalino I, Montagna C, et al. Diagnosis of Coxiella burnetii-related abortion in Italian domestic ruminants using single-tube nested PCR. *Vet Microbiol.* 2006;**118**(1-2):101–6. doi: 10.1016/j.vetmic.2006.06.023. [PubMed: 16891064]
20. Willems H, Thiele D, Frolich-Ritter R, Krauss H. Detection of Coxiella burnetii in cow's milk using the polymerase chain reaction (PCR). *Zentralbl Veterinarmed B.* 1994;**41**(9):580–7. [PubMed: 7740857]
21. Hoover TA, Vodkin MH, Williams JC. A Coxiella burnetti repeated DNA element resembling a bacterial insertion sequence. *J Bacteriol.* 1992;**174**(17):5540–8. [PubMed: 1324903]
22. Günaydin E, Müştak HK, Sareyyüpoğlu B, Ata Z. PCR Detection of Coxiella burnetii in Fetal Abomasal Contents of Ruminants. *Kafkas Universitesi Veteriner Fakultesi Dergisi.* 2015;**21**(1):69–73.
23. Pritchard GC, Smith RP, Errington J, Hannon S, Jones RM, Mearns R. Prevalence of Coxiella burnetii in livestock abortion material using PCR. *Vet Rec.* 2011;**169**(15):391. doi: 10.1136/vr.d4693. [PubMed: 21900261]
24. Cantas H, Muwonge A, Sareyyupoglu B, Yardimci H, Skjerve E. Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus. *BMC Vet Res.* 2011;**7**:13. doi: 10.1186/1746-6148-7-13. [PubMed: 21414196]
25. Berri M, Rousset E, Champion JL, Russo P, Rodolakis A. Goats may experience reproductive failures and shed Coxiella burnetii at two successive parturitions after a Q fever infection. *Res Vet Sci.* 2007;**83**(1):47–52. doi: 10.1016/j.rvsc.2006.11.001. [PubMed: 17187835]
26. Frazier ME, Mallavia LP, Samuel JE, Baca OG. DNA probes for the identification of Coxiella burnetti strains. *Ann N Y Acad Sci.* 1990;**590**:445–58. [PubMed: 2378470]
27. Lorenz H, Jager C, Willems H, Baljer G. PCR detection of Coxiella burnetii from different clinical specimens, especially bovine milk, on the basis of DNA preparation with a silica matrix. *Appl Environ Microbiol.* 1998;**64**(11):4234–7. [PubMed: 9797270]
28. Muramatsu Y, Maruyama M, Yanase T, Ueno H, Morita C. Improved method for preparation of samples for the polymerase chain reaction for detection of Coxiella burnetii in milk using immunomagnetic separation. *Veterinary Microbiology.* 1996;**51**(1-2):179–85. doi: 10.1016/0378-1135(95)00203-0. [PubMed: 8828134]
29. Yuasa Y, Yoshiie K, Takasaki T, Yoshida H, Oda H. Retrospective survey of chronic Q fever in Japan by using PCR to detect Coxiella burnetii DNA in paraffin-embedded clinical samples. *J Clin Microbiol.* 1996;**34**(4):824–7. [PubMed: 8815091]
30. Brom RV, Engelen EV, Roest HI, Hoek WV, Vellema P. Coxiella burnetii infections in sheep or goats: an opinionated review. *Vet Microbiol.* 2015. doi: 10.1016/j.vetmic.2015.07.011. [PubMed: 26315774]
31. van der Hoek W, van de Kassteele J, Bom B, de Bruin A, Dijkstra F, Schimmer B, et al. Smooth incidence maps give valuable insight into Q fever outbreaks in The Netherlands. *Geospat Health.* 2012;**7**(1):127–34. doi: 10.4081/gh.2012.111. [PubMed: 23242690]
32. Hatchette TF, Hudson RC, Schlech WF, Campbell NA, Hatchette JE, Ratnam S, et al. Goat-associated Q fever: a new disease in Newfoundland. *Emerg Infect Dis.* 2001;**7**(3):413–9. doi: 10.3201/eid0703.010308. [PubMed: 11384518]
33. Schimmer B, Morroy G, Dijkstra F, Schneeberger PM, Weers-Pothoff G, Timen A, et al. Large ongoing Q fever outbreak in the south of The Netherlands, 2008. *Euro Surveill.* 2008;**13**(31). [PubMed: 18761906]
34. Rousset E, Berri M, Durand B, Dufour P, Prigent M, Delcroix T, et al. Coxiella burnetii shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. *Appl Environ Microbiol.* 2009;**75**(2):428–33. doi: 10.1128/AEM.00690-08. [PubMed: 19011054]
35. Maurin M, Raoult D. Q fever. *Clin Microbiol Rev.* 1999;**12**(4):518–53. [PubMed: 10515901]
36. Porter SR, Czaplicki G, Mainil J, Guatteo R, Saegerman C. Q Fever: current state of knowledge and perspectives of research of a neglected zoonosis. *Int J Microbiol.* 2011;**2011**:248418. doi: 10.1155/2011/248418. [PubMed: 22194752]
37. Berri M, Souriau A, Crosby M, Rodolakis A. Shedding of Coxiella burnetii in ewes in two pregnancies following an episode of Coxiella abortion in a sheep flock. *Vet Microbiol.* 2002;**85**(1):55–60. [PubMed: 11792492]

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