African Journal of Microbiology Research Vol. 6(27), pp. 5594-5600, 19 July, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1266
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Prevalence study of *Coxiella burnetii* in aborted fetuses of small ruminants in various partum and seasons in Iran

Farhad Safarpoor Dehkordi[1]* and Mojtaba Salari Rafsanjani[2]

[1]Young Researchers Club, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
[2]Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

Accepted 9 June, 2012

Query (Q) fever is an ubiquitous zoonosis caused by *Coxiella burnetii*, an obligate intracellular rickettsial organism that cause abortion and stillbirth in ruminants. The prevalence of *C. burnetii* in Iran is essentially unknown. Its traditional diagnosis is based on culture, serology and conventional polymerase chain reaction (PCR). In this present study, for more sensitive and accurate detection and prevalence's determination of *C. burnetii* in aborted Ovine and Caprine fetuses, the nested and real-time PCR methods are recommended. 98 (12.53%) and 122 (16.39%) out of 782 and 744 Ovine and Caprine aborted fetuses, were positive for presence of *C. burnetii* by nested PCR, respectively. After real-time PCR, it was recognized that 121 (15.47%) and 152 (20.43%) samples were positive for *C. burnetii* in Ovine and Caprine aborted fetuses, respectively. Results indicated that the real-time PCR was 7 times more sensitive than the nested PCR. Statistical analysis showed significant differences about P < 0.01 between presence of *C. burnetii* in aborted Ovine and Caprine fetuses by both nested and real-time PCR assays and P < 0.05 between ability of nested and real-time PCR for detection of *C. burnetii*. The Ct values obtained from real-time PCR had significant differences of about P < 0.01 for presence of *C. burnetii* between aborted Ovine and Caprine fetuses. Our results indicated that Caprine is more sensitive than Ovine to *C. burnetii*'s abortion. Khozestan and Gilan have the highest, and Khorasan and Sistan va balochestan provinces have the lowest prevalence of *C. burnetii*, respectively. To our knowledge, this study is the first prevalence report of direct identification of *C. burnetii* in aborted Ovine and Caprine fetuses by evaluation of nested and real-time PCR assays in Iran. This study showed that the nested PCR for detecting *C. burnetii* are technically time-consuming and labor-intensive.

Key words: Prevalence study, *Coxiella burnetii*, small ruminant fetuses, various seasons, various partum.

INTRODUCTION

Coxiella burnetii is a strict fastidious obligate intracellular Gram-negative bacterium similar to rickettsia, which is a causative agent of an important ubiquitous worldwide zoonotic infectious disease named coxiellosis or query fever (Q fever) (Raoult et al., 2005). Disease can transmitted between animals and human by reservoirs including several species of mammals, birds, and arthropods, ticks, mites, fleas, lice and flies; but domestic and companion animals such as bovine, ovine, caprine and pets are the most important sources of human infection (Dupuis et al., 1987; Rauch et al., 1987; Hatchette et al., 2001). Human infections have been reported mainly in persons handling infected animals and their products (Armengaud et al., 1996).

Cattle and small ruminant, when infected, shed the desiccation-resistant organisms in urine, faeces, milk, and, especially, in material getting out during abortion or

*Corresponding author. E-mail: Dr.Farhads@yahoo.com. Tel: 98 9365819491. Fax: 98 3813381892.

parturition (Arricau-Bouvery and Rodolakis, 2005; Foley et al., 2003; Fournier et al., 1998). High concentrations of *C. burnetii* are found in the placentas of infected animals (Foley et al., 2003).

Coxiellosis occurs during late pregnancy (about 15 days before term), leading to abortion in small ruminants and stillbirth in cattle (Fournier et al., 1998).

Because *C. burnetii* is highly resistant to physical as well as chemical agents, it is difficult to control once it is disseminated in the environment. Therefore *C. burnetii* with these economic losses, needs accurate and sensitive diagnostic methods for rapid identification and elimination of persistent carriers in the herds. There are various methods for diagnosis of *C. burnetii* such as cell culture, serological and molecular methods. Cell culture method usually is both time consuming and hazardous.

The diagnosis of *C. burnetii* by some serological tests, which cannot be specific and sensitive enough due to cross-reaction (Rousset et al., 2009) especially in samples from areas with a low or subclinical prevalence of Coxiellosis. In addition, serological diagnostic methods are retrospective due to the time-frame for seroconversion (3 to 4 weeks post infection), rendering them useless for timely treatment. On the other hand, antibodies against *C. burnetii* may not appear until late into the course of the disease, and it is often difficult to make an early diagnosis based solely on serology for animal which present early. Since early diagnosis would be helpful for treatment of animals. In these years, DNA-based methods such as polymerase chain reaction (PCR), nested PCR and real-time PCR as safe and useful methods have been successfully used for detection of *C. burnetii* in clinical specimens (Berri et al., 2000; Kato et al., 1988; Klee et al., 2006). The ability to detect and quantify *C. burnetii* DNA by nested and real-time PCR has dramatically enhanced diagnostic and study approaches.

Although there have been a significant number of abortions in domestic animals in Iran, there is no study on reproductive problems and the causes of abortions in ruminants in Iran in the literature so far.

So, the two-fold purpose of the current study were to determine the prevalence rate of *C. burnetii* in aborted ovine and caprine fetuses in Iran, and evaluation of nested and real-time PCR assays to detection of *C. burnetii* in abomasal contents of aborted fetuses.

MATERIALS AND METHODS

Samples

From January 2010 to January 2011 (in various seasons of the year), a total of 782 ovine and 744 caprine aborted fetuses were collected from 108 commercial dairy herds including 60 ovine and 48 caprine of ten provinces of different parts of Iran (Table 1). In these areas of Iran, approximately 105 ovine and 72 caprine dairy herds are found. These samples were collected randomly from first, second, third and forth and top four partum ovine and caprine, and had only abomasal contents of aborted fetuses that were collected under sterile conditions and were immediately transported to the laboratory in a cooler with ice packs. The number of animal's partum were questioned from their owners. All abomasal content samples were kept at -20℃ until processing. In this study, in order to to avoid *C. burnetii* contamination during the necropsies of the fetuses in a farm, and between farms, 10 ml of abomasal contents of aborted fetuses were collected by 21G sterile needle in a free environment, away from animal and humans, and in the opposite direction of the wind. After sampling, fetuses and the contaminated materials were buried in a pit with 2 m depth and were covered with lime. This process was performed for each farm.

DNA extraction

C. burnetii DNA was extracted using a genomic DNA purification kit (Invitrogen, Paisley, U.K.) according to the manufacturer's instruction, and the total DNA was measured at 260 nm optical density according to the method described by previous study (Sambrook and Russell, 2001). In this study, DNA extraction was performed directly from 200 µl of abomasal contents. In this study, to rule out possible contamination between samples, distilled water was used as a negative control in DNA extraction and PCR assay

Nested PCR assay

All oligonucleotide primers were obtained from a commercial source (Cinna Gen, Iran). The nested PCR assay used to screen for *C. burnetii* in aborted fetuses was designed from the nucleotide sequence of the com1 gene encoding a 27-kD outer membrane protein (OMP) as previously described (Zhang et al., 1998) and the amplification was carried according to the method described elsewhere (Fretz et al., 2007). The PCR assay was performed at 94℃ for 4 min and then for 30 cycles of 94℃ for 1 min, 56℃ for 1 min, and 72℃ for 1 min in a DNA thermal cycler (ASTEC, Fukuoka, Japan). In the second amplification, the PCR assay was performed at 95℃ for 4 min and then for 30 cycles of 94℃ for 1 min, 57℃ for 1 min, and 72℃ for 1 min.

Gel electrophoresis

The PCR-amplified products (OMP1-OMP2: 501 bp; OMP3-OMP4: 438 bp) were examined by electrophoresis in a 1.5% agarose gel, stained with a 1% solution of ethidium bromide, and examined under UV illumination. In this study, *C. burnetii* DNA (Serial Number: 3154; Genekam Biotechnology AG, Duisburg, Germany) and DNase free water were used as the positive and negative controls, respectively.

Real-time PCR assay

Each abomasal content sample was tested using the commercial kit (targeting the repetitive transposon-like region of *C. burnetii*), LSI Taqvet *C. burnetii* (Laboratoire Service International, Lissieu, France) assay, according to the manufacturer's instructions. The negative control sample used was DNase RNase free water. The external positive control used was a solution containing 10^5 *Coxiella burnetii*/ml (provided by UR INRA IASP, Nouzilly, France). All real-time PCRs reactions were carried out using a RotorGene 6000 instrument (Corbett Research). For positive samples (having a typical amplification curve), the results are given in Ct (cycle threshold) values. Only the samples presenting a typical amplification curve with a Ct below 40 were considered positive.

The primer set consisted of primers trans-f (5′-

Table 1. distribution of *C. burnetii* in ten provinces of Iran using nested PCR and real-time PCR.

Province	No. herds in the study region		No. herds studied		No. samples per herd		No. fetuses samples		Nested PCR (%)		Real-Time PCR (%)	
	Ovine	Caprine	Ovine	Caprine	Ovine	Caprine	Ovine	Caprine	Ovine	Caprine	Ovine	Caprine
Isfahan	13	9	8	5	5 - 12	4 - 10	89	81	5 (5.61)	10 (12.34)	6 (6.74)	12 (14.81)
Gorgan	5	3	2	1	5 - 10	4 - 9	57	52	7 (12.28)	14 (26.92)	10 (17.54)	27(29.67)
Khozestan	11	8	5	4	5 - 8	4 - 8	83	77	18 (21.68)	21 (27.27)	22 (26.5)	25 (32.46)
Khorasan	11	5	5	3	4 - 10	3 - 11	78	70	2 (2.56)	11 (15.71)	3 (3.84)	13 (18.57)
Sistan va balochestan	7	6	3	3	4 - 8	3 - 9	71	75	3 (4.22)	4 (5.33)	5 (7.04)	6 (8)
Kerman	11	3	6	1	4 - 7	3 - 5	79	68	6 (7.59)	5 (7.35)	7 (8.86)	6 (8.82)
Gilan	16	14	10	8	3 - 8	2 - 7	92	91	19 (20.65)	23 (25.27)	23 (25)	22 (42.3)
Fars	19	15	13	9	3 - 7	2 - 5	103	96	14 (13.59)	7 (7.29)	16 (15.53)	9 (9.37)
Kordestan	9	5	2	2	2 - 5	2 - 4	75	64	13 (17.33)	15 (23.43)	16 (21.33)	18 (28.12)
Ilam	3	4	2	2	2 - 4	2 - 4	55	72	11 (20)	12 (16.66)	13 (23.63)	14 (19.44)
Total	105	72	56	38	2 - 12	2 - 11	782	744	98 (12.53)	122 (16.39)	121 (15.47)	152(20.43)

This study showed that *C. burnetii* in caprine, has a more severe effects than ovine.

GGGTAAAACGGTGGAACA ACA-3′) and trans-r (5′-ACAACCCCCGAATCTCATTG-3′). The internal probe trans-p (5′-AACGATCGCGTATCTTTAACAGCGCTTG-3′) was labeled with the reporter dye5-carboxyfluoroscein (FAM) on the 5′ end and the quencher dye N′, N′, N′, N′-tetramethyl-6-carboxyrhodamine (TAMRA) at the 3′ end.

Sensitivity of nested and real-time PCR assays

Solutions of purified *C. burnetii* were prepared ranging from 1×10^6 to 1 microorganism/100 µl. 200 µl of the solution at microorganism concentration were used, and the extracted DNA was dissolved in 100 µl of distilled water. 3 µl of the DNA solution were used in the nested and real-time PCR assays. Results are shown as number of microorganisms in one PCR-tube (microorganisms/PCR-tube) and in 200 µl of sample (microorganisms/sample).

Statistical analysis

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 18.0 statistical software (SPSS Inc., Chicago, IL, USA), ANOVA test analysis were performed and differences were considered significant at values of $P < 0.05$.

RESULTS

Results indicated that 98 out of 782 aborted ovine fetuses (12.53%) and 122 out of 744 aborted caprine fetuses (16.39%) were positive for presence of *C. burnetii* by nested PCR (Table 1).

After real-time PCR, from a total of 782 and 744 aborted ovine and caprine fetuses, *C. burnetii* gene were distinguished in 121 (15.47%) and 152 (20.43%) samples, respectively (Table 1). Our results showed that all of the herds that were collected for studied were infected for presence of *C. burnetii*. Therefore, from the total number of 105 ovine and 72 caprine dairy herds in these areas of Iran, 56 (53.33%) and 38 (52.77%) herds were infected, respectively (Table 1).

Sensitivity was compared between the nested and real-time PCR assays in the detection of *C. burnetii* DNA. The nested PCR assay with two pairs of primers, OMP1 and 2, and OMP3 and 4 detected 63 microorganisms/PCR-tube, equivalent to 2 × 103 microorganisms/sample, whereas the real-time PCR assay with the trans-f (5′-GGGTAAAACGGTGGAACAACA-3′) and trans-r (5′-ACAACCCCCGAATCTCATTG-3′) primers, detected 42 microorganisms/PCR-tube equivalent to 2×10^2 microorganisms/sample (data not shown), indicating that the real-time PCR assay was 1.5 times more sensitive than the nested PCR assay.

Statistical analysis showed significant differences about $P < 0.01$ between aborted ovine and caprine fetuses for presence of *C. burnetii* by both nested and real-time PCR assays and $P < 0.05$ between nested PCR and real-time PCR for detection of *C. burnetii*. In the other hand, The Ct values which obtained from real-time PCR had

Table 2. Distribution of *C. burnetii* in aborted ovine and caprine fetuses in various months of the year.

Species	No. of positive samples with real-time PCR (%)	Dec - Feb (%)	March - May (%)	Jun - Aug (%)	Sept - Nov (%)
Ovine	121 (15.47)	8 (6.61)	90 (74.38)	20 (16.52)	3 (2.47)
Caprine	152 (20.43)	9 (5.92)	123 (80.92)	16 (10.52)	4 (2.63)
Total	273 (17.88)	17 (6.22)	213 (78.02)	36 (13.18)	7 (2.56)

Table 3. Distribution of *C. burnetii* in various partums of ovine and caprine aborted fetuses.

Species	No. of positive samples with real-time PCR (%)	First partum (%)	Second partum (%)	Third partum (%)	Fourth and top four partum (%)
Ovine	121 (15.47)	71 (58.67)	32 (26.44)	10 (8.26)	8 (6.61)
Caprine	152 (20.43)	129 (84.86)	14 (9.21)	6 (3.94)	3 (1.97)
Total	273 (17.88)	200 (73.26)	46 (16.84)	16 (5.86)	11(4.02)

significant differences about $P < 0.01$ between aborted ovine and caprine fetuses.

So, this study showed the higher accuracy and sensitivity of real-time PCR than nested PCR to detection of *C. burnetii* in aborted ovine and caprine fetuses. Therefore, the prevalence rates of *C. burnetii* in aborted ovine and caprine fetuses are 12.78 and 19.08%, respectively.

In this present study, Khozestan (26.5%) and Gilan (42.3%) provinces have the highest and Khorasan (3.84%) and Sistan va balochestan (8%) provinces have the lowest prevalence rates of *C. burnetii* in aborted ovine and caprine fetuses in Iran, respectively (Table 1). Our results indicated that caprine is more sensitive than ovine to *C. burnetii*'s abortion (20.43% in Caprine versus 15.47% in Ovine).

Our results showed that the prevalence of *C. burnetii* in winter (December to February), spring (March to May), summer (June to August) and autumn (September to November) seasons of the year were 6.61, 74.38, 16.52 and 2.47% in ovine and 5.92, 80.92, 10.52 and 2.63% in caprine, respectively (Table 2). Statistical analysis showed significant differences ($p < 0.05$) between incidences of *C. burnetii*'s abortion in spring with winter and autumn seasons in both ovine and caprine aborted fetuses. Therefore, our study showed that *C. burnetii*'s abortion in both ovine and caprine species occurred mainly in spring (March to May) season.

This present study showed that the majority of *C. burnetii*'s isolations were from first and second partum animals (Table 2). From the total, 121 ovine and 152 caprine aborted fetuses were positive for *C. burnetii*, 71 (58.67%) and 129 (84.86%) were positive in first partum, 32 (26.44%) and 14 (9.21%) were positive in second partum, 10 (8.26%) and 6 (3.94%) were positive in third partum and finally 8 (6.61%) and 3 (1.97%) were positive in forth and top four partum (Table 3). These results showed that first partum ovine and caprine had a highest susceptibility to abortion with *C. burnetii*. Statistical analysis showed significant differences ($p < 0.05$) between incidences of *C. burnetii*'s abortion in first partum animals with third and forth and top four partum ovine and caprine species. In total, results showed the high prevalence rate of *C. burnetii* in animals of Iran.

DISCUSSION

In the majority of cases, *C. burnetii*'s abortion occurs at the end of gestation without specific clinical signs until abortion is imminent, as observed with brucellosis or chlamydiosis. Aborted fetuses appear normal but infected placentas exhibit intercotyledonary fibrous thickening and discolored exudates, which are not specific to Q fever. A severe inflammatory response is observed in the myometrium and the stroma adjacent to the placentomal area during gestation in goats.

Because *C. burnetii* may be shed by other routes such as milk, faeces, urine, placenta, or birth fluids, testing animal based on only abomasal contents of aborted fetus samples can lead to misclassifying the status of the animal (Guatteo et al., 2006). In fact, the differences between the prevalence of *C. burnetii* in ovine, and caprine abomasal contents of aborted fetus samples found in this study may be because of the different routes of shedding *C. burnetii* present in these animals. Ovine shed *C. burnetii* mainly in faeces and vaginal mucus, whereas caprine excrete *C. burnetii* in their vaginal discharges, faeces, and milk (Rodolakis et al., 2007). Furthermore, the infected animals may not persistently shed *C. burnetii*. Shedding of *C. burnetii* by infected animals occurs mainly during parturition and lactation. Therefore, detection of *C. burnetii* in abomasal contents of ovine and caprine aborted fetuses do not depend on the sampling time. In addition to the aforementioned, abomasal contents of aborted fetuses contain all of the vaginal, placental and even birth fluids and it is an

advantage of abomasal content samples than urine, faeces and even milk samples for detection of *C. burnetii*.

In addition to the route of infection, the inoculums size affects the expression of *C. burnetii* infection.

The *C. burnetii*'s abortion rate can range from 3 to 80% of pregnant animals (Berri et al., 2000; Palmer et al., 1983; Zeman et al., 1989). High abortion rates are rarely observed, except in some caprine herds (Palmer et al., 1983). Often, the number of animals that abort in the flock may not be enough to alert the farmer, and human clinical cases often reveal the infection of the flock (Berri et al., 2000). Ewes shed more and longer vaginal discharges than goats, and can shed bacteria at subsequent pregnancies (Berri et al., 2003).

The epidemiology of Q-fever in Iran is essentially unknown, and to the authors' knowledge, the prevalence rate of *C. burnetii* in ruminant's aborted fetuses in Iran has never been reported but there are some studies that reported detection of *C. burnetii* in various clinical samples in Iran (Khalili et al., 2010; Khalili and Sakhaee, 2009).

The prevalence rate of *C. burnetii* in aborted ovine in Iran (15.47%) is higher than in northern Spain (9%) (Oporto et al., 2006) and Italy (10%) (Masala et al., 2004) but is lower than Netherlands (up to 80%) (Roest et al., 2011) and the prevalence rates of *C. burnetii* in aborted caprine fetuses in Iran (20.43%) are higher than United Kingdom (25%) (Jones et al., 2010) and lower than Netherlands (up to 80%) (Roest et al., 2011) and Italy (21.5%) (Parisi et al., 2006).

This present study showed that nested PCR and real-time PCR assays can be used extensively as fast, safe and accurate diagnostic methods to detection of *C. burnetii* in aborted ovine and caprine fetuses but the real-time PCR assay is faster and more sensitive and accurate than nested PCR to detection of *C. burnetii*. In our study, the higher sensitivity shown by real-time PCR technique compared to nested-PCR could be due to the gen target used, LSI gen is used in real-time PCR (gen with multiple copies), whereas in nested-PCR is OMP gene (one copy). In addition, the real-time PCR assay that was used in this study is more accurate, sensitive and faster than conventional PCR in detection of *C. burnetii*. Besides, the real-time PCR assay has some advantages compared to the nested PCR; it is an important diagnostic tool yielding reliable and reproducible results, does not require post-PCR analysis (gel electrophoresis, hybridization), and the risk of cross contamination is limited compared to nested PCR method, but the real-time PCR is more expensive than nested PCR. This study showed that the nested PCR method for detecting *C. burnetii* is technically time-consuming and labor-intensive than real-time PCR assay. To our knowledge, in the majority of cases, placenta is a choice sample for diagnosis of Q fever abortion but in some conditions including failure to comply, hygiene in sampling and in polluted environments, easily be contaminated with other pathogens. In the other hand we want to show the contamination rate of abomasal contents of aborted fetuses with this *C. burnetii*. Therefore, we used the abomasal contents aborted fetuses instead of placenta for detection of *C. burnetii*.

Previous studies suggested the high sensitivity, specificity and accuracy of molecular methods such as PCR (Arricau-Bouvery et al., 2006), nested PCR (Fretz et al., 2007), PCR-Enzyme linked immune sorbent assay (PCR-ELISA) (Muramatsu et al., 1997) and real-time PCR (Guatteo et al., 2007) to detection of *C. burnetii* in clinical specimens.

Since, PCR has been developed for the detection of *C. burnetii* for a wide variety of clinical samples such as serum (Zhang et al., 1998), blood (Kato et al., 1988), urine (Vaidya et al., 2008), faeces (Berri et al., 2000), milk (Willems et al., 1994) vaginal tissue (Cairns et al., 2007) and semen (Milazzo et al., 2001).

To our knowledge, for diagnosis of Q fever abortion, placenta and vaginal mucus from aborted sheep and goats are the main samples; but in this study, we tried to used from the abomasal contents of aborted foetuses as another sources of *C. burnetii* in animals with abortions.

To our knowledge, probably higher presences of ticks in warmer and more humid seasons of the year is the main reason for higher incidence of *C. burnetii*'s abortions in March to May (spring season) months of this present study. Previous study in Cyprus (Cantas et al., 2011) showed that presences of ticks and carnivores in farm were risk factors but good hygiene was found to be a protective factor to *C. burnetii* abortions and it showed that abortion with *C. burnetii* had a seasonal pattern and this finding was similar with our results but this study in Cyprus showed that the highest occurrence was experienced in October which gradually declined to the lowest in December while our study showed that 74.38 and 80.92% of aborted cases respectively in ovine and caprine occurred between March to May. To our knowledge, this change of season could have a direct effect on tick prevalence which then indirectly affects the occurrence of the disease in this region. In addition to Cyprus and our study in Iran, the seasonal variation of C. *burnetii* has previously been documented in Japan (Yanase et al., 1997). This study on Japan showed that the number of antibody-positive animals and their antibody titers were significantly elevated in winter and decreased in summer. A study on human showed that seasonality occurrence of *C. burnetii* has been seen to occur in spring following the outdoor lambing of sheep in southern Germany (Hellenbrand et al., 2001). In addition to seasonal patterns for abortions caused by *C. burnetii*, our study for the first time in the world showed that incidence of abortions caused by this bacterium is highly dependent to animal's partum. Our results showed that the majority of cases occurred in first partum ovine and caprine (58.67 and 84.86%, respectively). On the other hand, the first partum animals are the most sensitive group

to abort with *C. burnetii*. To our knowledge, maybe low uterine growth and high stress of parturition and therefore severe reduce in the levels of body immunity are the main reasons for this event.

Studies showed that *C. burnetii* has the ability to shed from various resources such as faeces, urine, milk, birth fluids and placenta (Guatteo et al., 2006), and testing animal based on only one of shedding routes can lead to misclassifying the status of the animal. Probably, the differences between the incidence of *C. burnetii* in ovine and caprine aborted fetuses which was found in our study may be because of the different routes of *C. burnetii*'s shedding present in these animals. Another reason is the more close contact of animals with infected ovine and caprine in our study region. On the other hand, the infected animals may not persistently shed *C. burnetii* and our study showed that shedding of bacterium had seasonal pattern.

Our results showed that the majority of abortions caused by *C. burnetii* occurred from March to May and to our knowledge this period of time coincided with lambing in Iran. Therefore, probably the main cause of abortions in ovine and caprine herds in Iran was close contact with infected ovine and caprine's labor discharges in lambing period. To the author's knowledge, only a few amount of bacterium is excreted in faeces and urine; and in the majority of cases, vaginal secretions and milk are the main sources of *C. burnetii*. Therefore, detection of bacteria in these two sources caused more success on isolation of *C. burnetii*. Mainly, abomasal contents of ovine and caprine aborted fetuses contain all of the vaginal, placental and even birth fluids, and it is an advantage of abomasal content samples than urine, faeces and even milk samples for detection of *C. burnetii*. To the author's knowledge, in addition to the routes of infection, the inoculums size affects the expression of *C. burnetii* infection.

Although no extensive prevalence study was undertaken, the results of this study indicate that the prevalence rates of *C. burnetii*'s abortions are high in ovine and caprine in Iran.

To our knowledge, this study is the first prevalence report of direct detection of *C. burnetii* in various partum and seasons in aborted ovine and caprine fetuses in Iran by evaluation of nested PCR and real-time PCR assays. This present study suggested that real-time PCR is more sensitive and more accurate than nested PCR and can be used as a safe, fast and thrust full diagnostic method for detection of *C. burnetii*'s in aborted fetuses.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Hassan Momtaz, Prof. Farhid Hemmatzadeh, Dr. E. Tajbakhsh and Dr. E. Rahimi of the Biotechnology Research Center, Islamic Azad University, Shahrekord for their important technical and clinical support. This work was supported by the Islamic Azad University, Shahrekord Branch, Iran. Grant no. 90/9025.

REFERENCES

Armengaud A, Kessalis N, Desenclos JC, Maillot E, Brousse P, Brouqui P (1996). Urban outbreak of Q fever, Briancon, France, March to June. Eur. Surveill. 2:12–13.

Arricau-Bouvery N, Hauck Y, Bejaoui A, Frangoulidis D, Bodier CC, Souriau A, Meyer H, Neubauer H, Rodolakis A, Vergnaud G (2006). Molecular characterization of *Coxiella burnetii* isolates by infrequent restriction site-PCR and MLVA typing. BMC Microbiol. 6:38.

Arricau-Bouvery N, Rodolakis A (2005). Is Q Fever an emerging or re-emerging zoonosis. Vet. Res. 36:327-349.

Berri M, Laroucau K, Rodolakis A (2000). 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. 72:285-293.

Berri M, Rousset E, Champion JL, Arricau-Bouvery N, Russo P, Pepin M, Rodolakis A (2003). Ovine manure used as a garden fertiliser is suspected to be a contamination source of two human Q fever cases. Vet. Rec. 153:269-270.

Cairns K, Brewer M, Lappin MR (2007). Prevalence of *Coxiella burnetii* DNA in vaginal and uterine samples from healthy cats of north-central Colorado. J. Feline Med. Surg. 9:196-201.

Cantas H, Muwonge A, Sareyyupoglu B, Yardimci H, Skjerve E (2011). Q fever abortions in ruminants and associated on-farm risk factors in northern Cyprus. BMC Vet. Res. 7:13.

Dupuis G, Petite J, Peter O, Vouilloz M (1987). An important outbreak of human Q fever in a Swiss Alpine valley. Int. J. Epidemiol. 16:282-87.

Foley JE, Biberstein EL, Hirsh DC (2003). *Rickettsiae: Rickettsia, Coxiella*, and *Orienta*. In: Hirsh DC, 2004. MacLachlan NJ, Walker RL (eds.). Veterinary Microbiology: Second Edition. Blackwell Publishing, pp. 250-252.

Fournier PE, Marrie TJ, Raoult D (1998). Diagnosis of Q fever. J. Clin. Microbiol. 36:1823-34.

Fretz R, Schaeren W, Tanner M, Baumgartner A (2007). Screening of various foodstuffs for occurrence of *Coxiella burnetii* in Switzerland. Int. J. Food Microbiol. 116:414-418.

Guatteo R, Beaudeau F, Joly A, Seegers H (2006). Shedding routs of *Coxiella burnetii* in dairy cows: implications for detection and control. Vet. Res. 37:827-833.

Guatteo R, Beaudeau F, Joly A, Seegers H (2006). Shedding routs of *Coxiella burnetii* in dairy cows: implications for detection and control. Vet. Res. 37:827-833.

Guatteo R, Beaudeau F, Joly A, Seegers H (2007). Assessing the within-herd prevalence of *Coxiella burnetii* milkshedder cows using a real-time PCR applied to bulk tank milk. Zoonoses Publ Health 54:191-194.

Hatchette TF, Hudson RC, Schlech WF, Campbell NA, Hatchette JE, Ratnam S, Raoult D, Donovan C, Marrie TJ (2001). Goat-associated Q fever: A new disease in Newfoundland. Emerg. Infect. Dis. 7:413-419.

Hellenbrand W, Breuer T, Petersen L (2001). Changing Epidemiology of Q Fever in Germany, 1947-1999. Emerg. Infect. Dis.7:789-796.

Jones RM, Twomey DF, Hannon S, Errington J, Pritchard GC, Sawyer J (2010). Detection of *Coxiella burnetii* in placenta and abortion samples from British ruminants using real-time PCR. Vet. Rec. 167:965-967.

Kato K, Arashima Y, Asai S, Furuya Y, Yoshida Y, Murakami M (1988). Detection of *Coxiella burnetii* specific DNA in blood samples from Japanese patients with chronic nonspecific symptoms by nested polymerase chain reaction. FEMS Immunol. Med. Microbiol. 21:139-144.

Khalili M, Sakhaee E (2009). An update on a serologic survey of Q fever in domestic animals in Iran. Am. J. Trop. Med. Hyg. 80:1031-1032.

Khalili M, Shahabi-Nejad N, Golchin M (2010). Q fever serology in febrile patients in Southeast Iran. Trans Roy S Trop. Med. Hyg. 104: 623-624.

Klee SR, Tycaka J, Ellerbrok H, Franz T, Linke S, Baljer G, Appel B

(2006). Highly sensitive real-time PCR for specific detection and quantification of *Coxiella burnetii.* BMC Microbiol. 6:2.
Masala G, Porcu R, Sanna G, Chessa G, Cillara G, Chisu V, Tola S (2004). Occurrence, distribution, and role in abortion of *Coxiella burnetii* in sheep and goats in Sardinia, Italy. Vet. Microbiol. 99:301-305.
Milazzo A, Hall R, Storm PA, Harris RJ, Winslow W, Marmion BP (2001). Sexually Transmitted Q Fever. Clin. Infect. Dis. 33:399-402.
Muramatsu Y, Yanase T, Okabayashi T, Ueno H, Morita C (1997). Detection of *Coxiella burnetii* in Cow's Milk by PCR–Enzyme-Linked Immunosorbent Assay Combined with a Novel Sample Preparation Method. Appl. Environ. Microbiol. 63:2142-2146.
Oporto B, Barandika JF, Hurtado A, Aduriz G, Moreno B, Garcia-Perez AL (2006). Incidence of ovine abortion by Coxiella burnetii in northern Spain. Ann. NY Acad. Sci. 1078:498-501.
Palmer NC, Kierstead M, Key DW, Williams JC, Peacock MG, Vellend H (1983). Placentitis and abortion in goats and sheep in Ontario caused by *Coxiella burnetii.* Can. Vet. J. 24:60-61.
Parisi A, Fraccalvieri R, Cafiero M (2006). Diagnosis of Coxiella burnetii-related abortion in Italian domestic ruminants using single-tube nested PCR. Vet. Microbiol. 118:101-106.
Raoult D, Marrie T, Mege J (2005). Natural history and pathophysiology of Q fever. Lancet. Infect. Dis. 5:219-226.
Rauch AM, Tanner M, Pacer RE, Barrett MJ, Brokopp CD, Schonberger LB (1987). Sheep-associated outbreak of Q fever Idaho. Arch. Int. Med. 147:341-344.
RodolakisA, Berri M, Héchard C, Caudron C, Souriau A, Bodier CC, Blanchard B, Camuset P, Devillechaise P, JC, Natorp J, Vadet P, Arricau-Bouvery N (2007). Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. J. Dairy Sci. 90:5352-5360.
Roest HI, Ruuls RC, Tilburg JJ, van der Hoek W, Vellema P, van Zijderveld FG, Klaassen CH, Raoult D (2011). Molecular pidemiology of *Coxiella burnetii* from Ruminants in Q Fever Outbreak, the Netherlands. Emerg. Infect. Dis. 17:668-675.
Rousset E, Berri M, Durand B, Dufour P, Prigent M, Delcroix T, Touratier A, Rodolakis A (2009). *Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Appl. Environ. Microbiol. 75:428-433.
Sambrook J, Russell DW (2001). Molecular Cloning: A Laboratory Manual, 3rd edition. Cold Spring Harbor Laboratory Press, New York, pp. 544-553.
Vaidya VM, Malik SVS, Kaur S, Kumar S, Barbuddhe SB (2008). Comparison of PCR, Immunofluorescence Assay, and Pathogen Isolation for Diagnosis of Q Fever in Humans with Spontaneous Abortions. J. Clin. Microbiol. 46:2038-2044
Willems H, Thiele D, Frohlish-Ritter R, Krauss H (1994). Detection of Coxiella burnetii in cow's milk using the PCR. Zentralbl. Veterinarmed. B. 41:580-587.
Yanase T, Muramatsu Y, Ueno H, Morita C (1997). Seasonal variations in the presence of antibodies against *Coxiella burnetii* in dairy cattle in Hokkaido. Jpn. J. Microbiol. Immunol. 41:73-75.
Zeman DH, Kirkbride CA, Leslie-Steen P, Duimstra JR (1989). Ovine abortion due to *Coxiella burnetti* infection. J. Vet. Diagn. Invest. 1:178-180.
Zhang GQ, Hotta A, Mizutani M, Ho T, Yamaguchi T, Fukushi H, Hirai K (1998). Clinical evaluation of a new PCR assay for detection of *Coxiella burnetii* in human serum samples. J. Clin. Microbiol. 36:77-80.