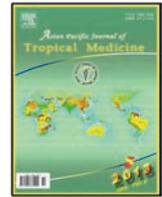




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Coxiella burnetii, the causative agent of Q fever in Saudi Arabia: molecular detection from camel and other domestic livestock

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ABSTRACT

Objective: To detect *Coxiella burnetii* (*C. burnetii*) DNA in clinical specimens from camel, goats, cattle and sheep in the Kingdom of Saudi Arabia. **Methods:** A total of 367 clinical samples including blood, milk, faeces and urine were collected from different livestock and subjected to PCR amplification using primers which amplify transposon-like region and transposase gene. **Results:** Positive amplification from both regions was obtained from camel, goats and cattle but not from sheep. A percentage of 10.8% samples yielded positive PCR amplification from both blood and milk, where 15 of 139 blood and 16 of 148 milk samples were positive. Faeces and urine showed higher percentages of positive samples reaching 40.8% and 23.8% respectively. **Conclusion:** The preferred route of shedding in camel appeared to be the faeces followed by urine, while that of goats appeared to be the faeces and that of the cattle appeared to be the milk.

1. Introduction

Q fever is a zoonotic disease caused by the intracellular bacterium *Coxiella burnetii* (*C. burnetii*), a member of the γ subgroup of proteobacteria[1]. It affects several animal species including mammals such as ruminants, equines, dogs, cats, non-human primates, wildlife (small and game animals), sea mammals as well as fish, amphibians and reptiles[2]. Ticks are considered as the natural primary reservoir of *C. burnetii* and are responsible for the spread of the infection in wild animals and sometimes its transmission to domestic animals[3]. Domestic livestock such as dairy

cattle, sheep, and goats are also known reservoirs of Q fever,

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and potentially incriminated as sources of outbreaks of human Q fever[4]. *C. burnetii* as an air pollutant constitutes an important source of infection to animals and humans; however, contaminated clinical materials and unpasteurized milk, can be an additional source of infection with Q fever[5]. The role of contaminated dust and aerosols derived from contaminated animal matrices from *C. burnetii*-positive dairy goat farms as a source of *C. burnetii* transmission to humans has been documented during the recent epidemic in the Netherlands[6–8].

In animals, the organism is mainly found in the reproductive system and may primarily cause abortion or infertility. In humans, however, the disease caused by *C. burnetii* is associated with acute flu-like illness, hepatitis, pneumonia and chronic endocarditis. The disease may affect different ages and is more frequent in men than in women. Mortality in chronic conditions may reach 1% to 11%[9].

Detection of *C. burnetii* DNA in animals can be achieved by the PCR in a wide range of clinical materials including; vaginal discharge, abortion products, faeces, milk, urine and blood. It has become increasingly common in diagnostic laboratories with PCR capabilities^[10,11]. Studies from several countries have been published recently on the detection of *C. burnetii* DNA in blood, milk, and other clinical samples from domestic as well as wild animals^[12–18].

Serological evidence of Q fever in Saudi Arabia was first reported in the 1960s among Riyadh inhabitants^[19,20], while no report was documented from animals till 2008^[21]. In 2012, Hussein and others reported antibodies to the disease from wildlife in Saudi Arabia^[22]. There is no report indicating that *C. burnetii* DNA was found in clinical materials from animals in the country. Therefore, in the present study, different clinical samples from serologically positive camels and other farm animals were subjected to PCR investigations to determine which animal species was more likely to shed *C. burnetii* and which route was the preferred shedding route for each animal species.

2. Materials and methods

2.1. Samples and DNA extraction

A total of 367 blood, milk, faecal and urine samples were collected from camels and other livestock which showed positive antibodies against *C. burnetii* when tested on ELISA (Table 1).

DNA was extracted from blood using the QIAGEN DNeasy blood and tissue kit (GmbH, Hilden, Germany) following the manufacturer's instructions.

Milk samples from lactating female camels, does, ewes and cows were kept at $-80\text{ }^{\circ}\text{C}$ until used. DNA was extracted as follows: a volume of 1 mL milk was centrifuged at 8 000 g for 60 min, the cream and milk layers were removed and the pellet was washed twice in distilled water; DNA was extracted using DNA extraction kit from QIAGEN using the QiaAmp mini kit (GmbH, Hilden, Germany). DNA from faecal samples from camels and goats was extracted using a commercial kit from Bioline (Bioline, Humber Rd, London NW2 6EW, UK). DNA from urine samples from camels was

extracted using the QiaAmp mini extraction kit (GmbH, Hilden, Germany). Briefly 1 mL of urine was centrifuged for 30 min and the supernatant was discarded and the sediment was used for DNA extraction. A total volume of $2.5\text{ }\mu\text{L}$ was used for PCR. All extraction procedures were made according to manufacturers' instructions.

2.2. *Coxiella burnetii* positive control DNA

Positive control DNA of *C. burnetii* was kindly provided by Professor Klaus Henning from the Institute of Epidemiology, Friedrich–Loeffler–Institute, Seestra ß e 55, 16868 Wusterhausen, Germany.

2.3. Polymerase chain reaction (PCR)

Amplification of the repetitive transposon–like region of *C. burnetii* was employed using 3 pairs of primers targeting the sequence. These primers include: IS111F1 ($5'–\text{TACTGGGTGTTGATATTGC}–3'$), IS111R1 ($5'–\text{CCGTTTCATCCGCGGTG}–3'$) which are designed to amplify 485–bp fragment of the htpAB–associated repetitive element (GenBank accession number M80806) and primers IS111F2 ($5'–\text{GTAAAGTGATCTACACGA}–3'$), and IS111R2 ($5'–\text{TTAACAGCGCTTGAACGT}–3'$) which are designed to amplify 260 bp of the original PCR resulting from using primers IS111F1 and IS111R1 in a multiplex PCR^[23,24]. The primers which amplify 448 bp product of the transposase gene of *C. burnetii* include CoxP4 ($\text{TTAAGGTGGGCTCGGTGGTGATGG}$, nucleotide positions 222–245 in GenBank accession M80806) and CoxM9 ($\text{GCTTCGTCCCGGTTCAACAATTGC}$, nucleotide position 669–648) were also used in this study^[25].

Each $25\text{ }\mu\text{L}$ reaction mixture was made of $5\text{ }\mu\text{L}$ of the PCR buffer (Bioline, UK), $0.2\text{ }\mu\text{L}$ of the taq polymerase (Bioline, UK), $1\text{ }\mu\text{L}$ of each of the four primers ($10\text{ pm}/\mu\text{L}$), IS111F1, IS111R1, IS111F2 and IS111R2, sterile distilled water, and $2\text{ }\mu\text{L}$ of DNA. The IS111F1 and IS111R1 primers, which were designed to amplify a 485–bp fragment of the htpAB–associated repetitive element, were used for the first amplification, and reamplification was performed using the IS111F2 and IS111R2 primers, which amplify a 260–bp fragment^[24]. Following an initial denaturation step at $95\text{ }^{\circ}\text{C}$ for 8 min, the rapid PCR program was made of 35 cycles of

Table 1

Samples collected from different animal species which were tested using PCR for the detection of *C. burnetii* DNA.

Animal Species	Blood	Milk	Faeces	Urine	Total
Camelus dromedarius	82	77	29	21	209
Capra hircus	38	29	20	0	87
Ovis aries	22	4	0	0	26
Bos taurus	7	38	0	0	45
Total	149	148	49	21	367

denaturation at 95 °C for 15 s, annealing at 52 °C for 5 s, and extension at 72 °C for 18 s. Reamplification or second round PCR was performed using 35 cycles of denaturation at 95 °C for 15 s, annealing at 48 °C for 5 s, and extension at 72 °C for 18 s. The amplification was completed by holding for 10 min at 68 °C to allow complete extension of the PCR products. Amplicons from the second amplification were separated by electrophoresis on 1% agarose gels and digital images were taken after staining gels using ethidium bromide and visualising the PCR products on transilluminator.

3. Results

Positive amplification was obtained, using the primers which amplify the repetitive transposon-like and transposase regions of *C. burnetii*, from blood, faeces, milk and urine of the camels investigated in the present study. Amplification was also obtained from blood and faecal but not from milk samples obtained from goats. From the cows samples amplification was obtained only from the milk and not from the blood. None of the samples collected from sheep revealed positive amplification for *C. burnetii* DNA (Table 2).

Out of 149 blood samples collected from different animal species, 15 samples (10.1%) showed positive amplification for *C. burnetii* DNA. Of these samples 13 were from camels while 2 from goats (Table 2). Out of 148 milk samples collected from different animal species, 16 samples (10.8%) yielded positive amplification for *C. burnetii* DNA, five of these samples were from camels while 11 were from cows. Faecal samples which were collected from 49 animals revealed positive PCR products from 20 samples (40.8%); eight of those were from camels whereas 12 were from goats. Urine samples ($n=21$) were collected from camels only and five (23.8%) of these samples revealed positive PCR products for *C. burnetii*.

Shedding of *C. burnetii* by the camels was found to be highest in faecal samples (27.6%) followed by urine (23.8%), then the blood (15.9%) and least in milk (6.5%). The highest percent detection of *C. burnetii* DNA from goats was found in the faeces (60%) and then in the blood (5.3%). In cows, *C. burnetii* DNA shedding was found only in 28.9% of the milk samples investigated.

4. Discussion

The present study is the first report on the direct detection of *C. burnetii* in camels, goats and cows in Saudi Arabia. The results indicate clearly that camels in Saudi Arabia may play an important role as a reservoir of *C. burnetii* and that they could be a significant source for the transmission of Q fever to humans.

C. burnetii has already been detected using different PCR methods in blood samples of infected camels in Iran[26], where partial 16S ribosomal RNA gene was amplified. But, to our knowledge, the present study is the first report worldwide of direct identification of *C. burnetii* by PCR in the milk, faeces and urine of naturally infected camels. Faecal material is known to contain several inhibitors of Taq polymerase[4,27]. However, in the present study the methods used for DNA extraction were successful and therefore DNA amplification from *C. burnetii* was obtained.

It appears that the camel is likely to harbor high concentrations of the organism and that it is capable of shedding it through milk, blood, faeces and urine. Q fever antibodies have been reported from camels in many countries in Africa, Asia and the Middle East[21, 28–30]. The only report which was published on the direct detection of *C. burnetii* DNA from camel was from Iran by Doosti and others[26], where partial DNA of 16S ribosomal RNA gene of *C. burnetii* was detected from the blood (10.8%). In the present study the DNA of the organism has been

Table 2

Different samples collected from camels, goats, sheep, and cows which were positive on ELISA and tested for the *C. burnetii* DNA using the polymerase chain reaction (PCR).

Animal species	Sample	No examined	No positive	No negative	Percentage positive (%)
Camelus dromedarius	Blood	82	13	69	15.9
	Milk	77	05	72	6.5
	Faeces	29	08	21	27.6
	Urine	21	05	16	23.8
Capra hircus	Blood	38	02	36	5.3
	Milk	29	00	29	0.0
	Faeces	20	12	08	60.0
Ovis aries	Blood	22	00	22	0.0
	Milk	04	00	04	0.0
Bos taurus	Blood	07	00	07	0.0
	Milk	38	11	27	28.9

demonstrated from urine (23.8%), faeces (27.6%), blood (15.9%) and milk (6.5%) using both transposon-like region and transposase gene. We have tested the presence of *C. burnetii* DNA in different clinical samples, because *C. burnetii* may be shed by other routes such as vaginal mucus, urine, faeces or birth fluids, hence testing animals on only blood samples can lead to misclassifying the status of the animal and misidentifying the route of excretion or discharge[27]. From the present study it appeared that the most suitable route of discharge for the organism in camel is the faeces followed by urine.

The differences between the prevalence of *C. burnetii* in bovine, ovine, and caprine milk samples found in some studies may be due to the fact that there are different routes of shedding of the organism in these animal species. It is generally believed that, in all ruminant species, shedding of *C. burnetii* could be related to parturition. It was found by Rodolakis and others[12], that this was not necessarily true and that the shedding of *C. burnetii* differed according to species and among the herds of the same species, except for ovine flocks. The main route of shedding by ovine was found to be the faeces and vaginal mucus while these routes were rare in bovine herds. Caprines were found to shed the organism via vaginal discharges, faeces and milk[12]. The absence of *C. burnetii* DNA from the sheep samples could be attributed to the fact that the organism in this animal species is shed primarily via vaginal mucus and faeces which have not been tested in the present study, and this probably confirms that milk and blood are not the preferred routes of discharge for *C. burnetii* in sheep. Another possible explanation is that shedding of the organism occurs intermittently in sheep and other species.

Molecular studies in goats and other animal species are limited due to lack of simple and sensitive detection tools. Shedding of *C. burnetii* in goats via vaginal mucus, faeces, and milk lasted for 1–5 weeks, 2–5 weeks and 1 day to 6 weeks respectively[31–33]. In the present study goats seemed to have shed *C. burnetii* through faeces and blood but not the milk, with the faeces showing a high rate of discharge. The absence of *C. burnetii* DNA from caprine milk samples confirms the fact that the main route of shedding in caprine is faeces and blood as has been reported earlier.

Detection of *C. burnetii* DNA in the milk of cows confirmed that milk may be the main route of transmission in this animal species. In a previous study by Barlow an association between *C. burnetii* shedding and subclinical mastitis in dairy cattle was reported[34]. Cows investigated in the present study may have had subclinical mastitis but it has not been detected.

Only techniques allowing the direct identification of *C. burnetii* shedders appear to be informative for assessing

the actual route of transmission of the infection. Hence, detection of *C. burnetii* in milk or blood significantly depends on the sampling time. The use of repeated sampling can reduce the likelihood of erroneously identifying herds as Q fever negative[35]. It is likely that the sample collection in the present study may have coincided with the shedding period for some individuals and not with others. Therefore, frequent sampling in affected herds is recommended in any epidemiological studies dealing with Q fever in goats.

In conclusion, this study reports epidemiological findings suggesting that camel, goat and cattle play a significant role in the transmission of *C. burnetii* to other animal species and to humans in Saudi Arabia. The preferred route of shedding of *C. burnetii* in camel appeared to be the faeces followed by urine, while that of goats appeared to be the faeces and that of the cattle appeared to be the milk.

Conflict of interest statement

We declare that we have no conflict of interest.

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