



Presence of *Coxiella burnetii* in Airborne Dust Samples from Goat and Sheep Farms in Kerman, Iran

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ARTICLE INFO

Article type:
Original Article

Article history:

Received: 9 Aug 2015
Revised: 16 Sep 2015
Accepted: 08 Oct 2015
Published: 15 Dec 2015

Keywords:

Coxiella burnetii, Dust,
PCR, Kerman, Iran.

ABSTRACT

Background: Q fever is a zoonotic disease caused by inhalation of the bacterium *Coxiella burnetii*. Ruminant livestock are common reservoirs for *C. burnetii*, and bacteria present in aerosols derived from the waste of infected animals can infect humans. *C. burnetii* is thought to infect humans primarily via airborne transmission.

Methods: 64 environmental swab samples were collected from 24 sheep and goat farms in Southeast Kerman province (Iran).

Results: In this study touchdown nested trans-PCR were used for detection of *C. burnetii* in environmental samples. We detected *C. burnetii* DNA in inhalable dust samples collected at 5 farms.

Conclusion: This first report in Iran highlighted presence of *C. burnetii* in dust originated from goat and sheep farms and that role in human infections with disseminating by wind.

- **Please cite this paper as:** Khalili M. Presence of *Coxiella burnetii* in Airborne Dust Samples from Goat and Sheep Farms in Kerman, Iran. *J Med Bacteriol.* 2015; **4** (5, 6): pp.19-23.

Introduction

Q fever is a zoonotic disease caused by the bacterium *C. burnetii*, which is found worldwide in many different animal species (1). *C. burnetii* transmitted by inhalation of aerosols contaminated, typically from the waste products of infected animals. Ruminant livestock commonly serve as reservoirs of *C. burnetii*, and goats, sheep, and cattle are the animals most often associated with Q fever outbreaks (2, 3). *C. burnetii* can be shed from urine, feces, and milk, but the highest numbers of *C. burnetii* isolates come from infected birth products. *C. burnetii* replicates to very high levels in the placental tissue of infected animals, and aerosols with high concentrations of *C. burnetii* can be generated during parturition (4), although it is not an endospore, the small-cell variant (SCV) form of *C. burnetii* is known to be very stable under a variety of conditions (5). It is suggested that *C. burnetii* can remain infectious for more than 40 months even under very unfavorable external conditions (6). The bacterium survives as spore stage on wool of sheep for 7-10 months at 15-20 °C, for more than 1 month at 4 °C on fresh meat, and for more than 40 months in dry milk powder at room temperature (7). *C. burnetii* is also extremely infectious for humans; 1-10 viable organisms suffice to induce an infection via the aerogenic route (2). The clinical manifestations of Q fever in humans are variable. Thus acute Q fever in humans usually manifests as an asymptomatic or mild flu-like disease with spontaneous recovery. In some people, the disease can lead to a chronic infection that can manifest years later, even in the absence of primary, acute Q fever symptoms (4). Urban outbreaks and cases with no known exposure or close proximity to livestock have been reported, as have non occupational exposures such as through a hobby farm (a small farm that is not a primary source of income) (8). The largest known reported Q fever outbreak involved approximately 4000 human cases and occurred during 2007–2010 in the Netherlands. This outbreak was linked to dairy goat farms near densely populated areas and

presumably involved human exposure via a windborne route (11) The available evidence obtained from various studies in the southeast of Iran (9, 10, 11), implies that Q fever is endemic in animals and humans and is considered an occupational disease in humans in Iran. The aim of the current study was to investigate presence of *C. burnetii* in inhalable dust samples from goat and sheep farms in Southeast Iran.

Materials and Methods

Samples

For each individual farm, 2-3 swabs from horizontal (dust-accumulating) surfaces, totally 64 environmental swab samples were collected from 24 sheep and goat farm in different counties located in the Kerman province (Southeast Iran) Sep 2014 to Sep 2015 (Figure 1).

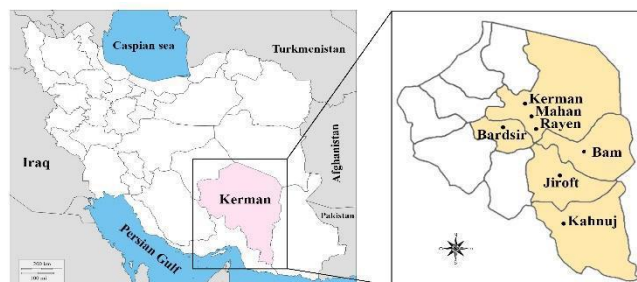


Figure 1. Sample collection area in Kerman Province.

DNA extraction

DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The extracted DNA stored at -20 °C until used.

PCR assay

To improve detection of *C. burnetii* DNA in environmental samples we used the nested trans-PCR assay. The primers Trans1, Trans2 and 261 F, 463 R internal primers were used as previously

described (12, 13). These primers were designed based on a repetitive, transposon-like element (Trans PCR) has proved to be highly specific and sensitive for the laboratory diagnosis of *C. burnetii* infections, as it detects even very few copies of a specific DNA sequence (14). The sequences of the primers used in the study are presented in Table 1.

Table 1. Sequences of the primers used in this study.

Protocol	Primer	Sequence	Gen	Amplicon	Reference
Trans PCR	Trans1	TA TGTATCCACCGTAGCCAGT	B1111	687	12
	Trans2	CCCAACAACACCTCCTTATTC			
Trans PCR	261F	GAGCGAACCATTGGTATCG	B1111	203	13
	463R	GAGCGAACCATTGGTATCG			

Controls

The DNA from the Nine Mile II (strain RSA 493) was used as a positive control, and sterile water was used as a negative control.

Results

C. burnetii DNA was detected in 5 swab samples in goat and sheep farms inhalable dust samples collected (Figure 2).

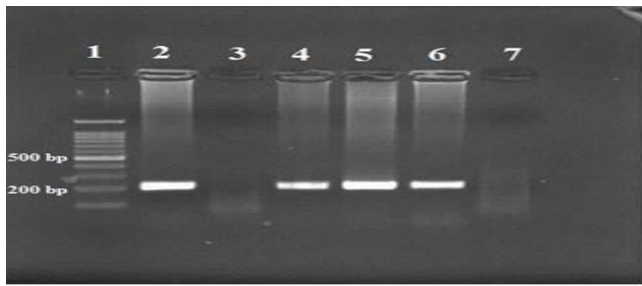


Figure 2. Nested Trans-PCR results: Lane 1; 100 bp Marker, 2; Nine Mile RSA 493, 3; NTC, 4-6; Positive samples, 7; negative sample.

Discussion

Evidence of environmental contamination was found in farm samples described in this study. Other studies have shown that *C. burnetii* can be detected in dust samples (15), soil samples (16) or

aerosols (17, 18) farm environmental samples (19, 20). Previous study in southeast Iran showed Q fever is an endemic phenomenon in this area and goats to have a significantly higher average seroprevalence of antibodies to *C. burnetii* than other species (21).

Based on the replication of *C. burnetii* to very high levels in the placenta of infected goats the highest organisms found in the birthing areas (20). Because *C. burnetii* is very resistant to heat and drought, the bacterium can be transmitted through contaminated aerosols (22) Therefore, the higher prevalence of Q fever in Central Iran could be ascribed to favorable climatic conditions for aerosol transmission of the bacterium in this area (8); also suggested aerosol spread as an important route of infection of the dairy goat farms in the Netherlands. Based on the recent climatic conditions in the Southeast Iran and our finding predicted, the aerosol transmission of Q fever could be the most important route of infection of sheep and goat flocks in the studied areas and that may be potential of human infection. It is a well-established fact that the exposure of the human respiratory system to high concentrations of airborne particulate matter (dust) can have significant adverse health effects. Desert storm activity is the most significant source of non-occupational dust exposure in arid and semiarid regions of the world (15). The Sistan region located in Southeastern Iran is a major dust source in southwest Asia, often producing intense dust storms that cover the southwest Afghanistan and Pakistan and southeast Iran. Particles from dust storms might also cover farm and grasslands to result in damage to crops and fill the rivers and water channels with aeolian material. After the extreme drought of 1999, the dust activity over Sistan appears to be increasing in both frequency and severity (26). Gilbert demonstrated that *C. burnetii* can spread to the environment from infected goats. *C. burnetii* found in the environment can remain viable and infectious.

In our study we have shown that *C. burnetii* can be detected in airborne dust samples that can be

inhaled by humans. This supports the general assertion that airborne transmission might indeed be a likely route of exposure, and it should be explored in more detail to understand the spread and transmission route of *C. burnetii* and the risk posed by *C. burnetii* for humans.

Acknowledgements

This research was financially supported by Iran National Science Foundation.

Conflict of interest

None declared conflicts of interest.

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