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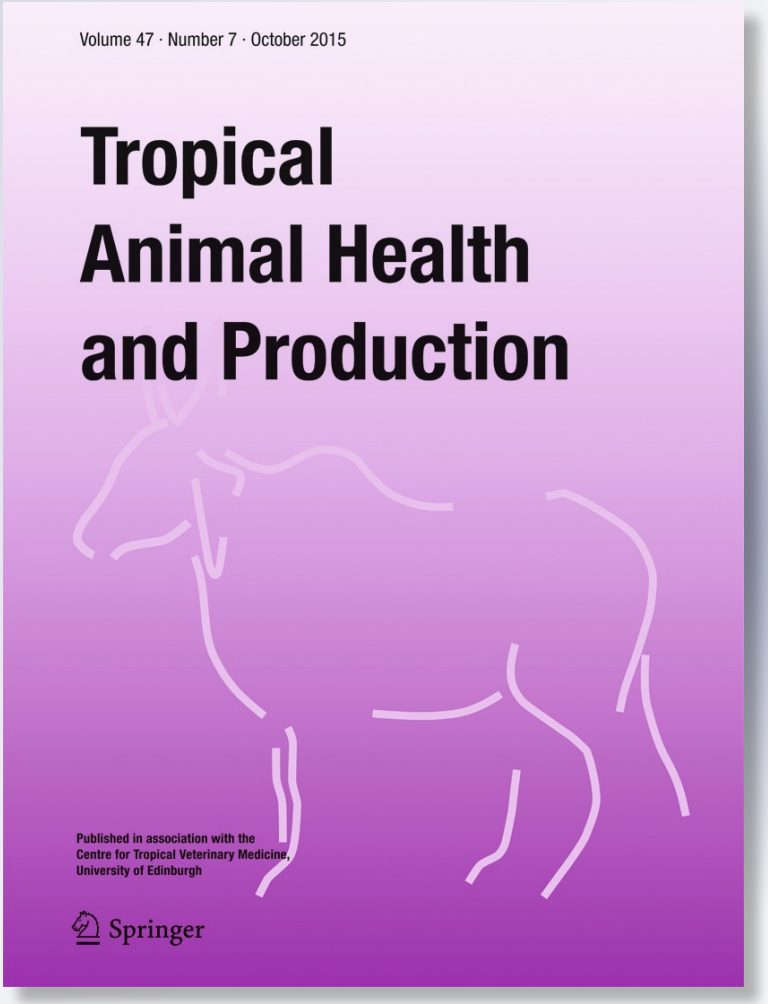

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REGULAR ARTICLES

Seroepidemiology of Q fever in one-humped camel population in northeast Iran

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Abstract *Coxiella burnetii*, an obligate intracellular bacterium, is the causative agent of important zoonotic Q fever. It is the etiological agent of coxiellosis or Q fever in animals and human. This seroepidemiological survey was conducted to determine the seroprevalence of coxiellosis in increasingly camel raised population in vast area of Khorasan (North, South, and Razavi) provinces, northeast Iran. Using cluster random sampling strategy, 167 camels in 11 counties were selected as serum samples. Sera were assayed for antibody against *C. burnetii* using a Q fever ELISA kit. Logistic regression model was used to insight the contributing risk factor(s) of Q fever in the study area. *C. burnetii* was widely distributed throughout the study area. Seroprevalence of *C. burnetii* at animal level was 28.7 % [(95 % confidence interval (CI): 21.83, 35.56)] for camel populations. The proportion of seropositivity for camels in the studied counties ranged from 0 to 63.6 %. Logistic regression model showed that age correlated with seroprevalence of coxiellosis at the individual level in camels ($P<0.05$). This study showed that a relatively high proportion of camels are seropositive to *C. burnetii*. Considering the economic, zoonotic, and public health importance of Q fever, percussion measures are to be implemented to prevent spreading of *C. burnetii* and zeroing the risk of Q fever in farm animals and human in this agro-ecologically and geopolitically important region.

Keywords Coxiellosis · Camel · NE Iran · Public health · Q fever · Seroepidemiology · Zoonosis

Introduction

Q fever is a highly contagious zoonotic disease caused by the intracellular pathogen *Coxiella burnetii*. It is highly pathogenic (i.e., only one organism can be enough to infect farm animals and humans (Ormsbee et al. 1978; Ruiz and Wolfe 2014; van Loenhout et al. 2015). A wide variety of animals can be infected with *Coxiella burnetii*, including ruminants, dogs, cats, non-human primates, reptiles, birds, fish, and even ticks (Norlander 2000; Tissot-Dupont et al. 2007; Tozer et al. 2013; O'Connor et al. 2015; Pimenta et al. 2015). Ruminants are the main reservoirs. *Coxiella burnetii* infection might be the cause of abortion, stillbirth, low birth weight, infertility, and heart failure in cattle, sheep, and goat (Marrie et al. 1996), leading to economic loss and public health risk. Infected animals and humans shed the bacterium through urogenital, gastrointestinal tracts and milk; it also notoriously spreads through inhalation of infected aerosol (Arricau-Bouvery and Rodolakis 2005; Tissot-Dupont et al. 2007; Schimmer et al. 2010; Tozer et al. 2013; Schimmer et al. 2014; O'Connor et al. 2015; Nusinovici et al. 2015; van den Brom et al. 2015), widening its transmission routs (i.e., aerosols, milk, and dairy products) and thus Q fever outbreak in rural and urban area.

Although the most common animals involved in the outbreaks of Q fever in human are sheep and goats (McQuiston and Childs 2002; Arricau-Bouvery and Rodolakis 2005), the involvement of camelids in this outbreak should also be taken

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into consideration (Mathur and Bhargava 1979; Ferrante and Dolan 1993; Doosti et al. 2014). In human, while the initial infection seems asymptomatic, prolonged fever, pneumonia, hepatitis, and cardiac involvement, especially endocarditis being the most clinical sign of the chronic form, are the main consequence of the acute form of Q fever (Angelakis and Raoult 2010; Ruiz and Wolfe 2014; van Loenhout et al. 2015).

Research has indicated the danger of *Coxiella burnetii* infection in camel farmers/workers, who are in close contact with dromedaries (Mathur and Bhargava 1979; Ferrante and Dolan 1993). Considering the zoonotic aspect of *Coxiella burnetii* infection and the recent occurrence of Q fever in humans in Afghanistan and Iran (Hartzell et al. 2007; Aronson 2008; Khalili et al. 2010; Bailey et al. 2011), the determination of seroprevalence of *Coxiella burnetii* in domesticated animals as hosts/reservoirs for infection in man has long been established. The dromedary is no exception. Study on the epidemiology of coxiellosis in Iranian camels is little. Agroecologically and geopolitically, the selected study zone is important, worldwide. Further, there is a great concern about increasingly camel application not only for sustainable medicinal meat and milk production but also for booming agrotourism industries in the study area, wherein the harsh environment and huge water shortage make it almost impossible to rear/produce farm animals other than camel. Also, in the study zone, many peacekeepers are relentlessly working to route out terror and bring peace/prosperity for the region, particularly Afghanistan, and zoonosis issue of Q fever is one of the main concerns for human. As such, seroepidemiological investigation of *Coxiella burnetii* in widely raised one-humped camel region, like Iran's porous long borders with Afghanistan, Pakistan, and Turkmenistan, where farm animals illegally enter Iran, seems necessary. In the present seroepidemiological study, we aimed to determine the seroprevalence of *Coxiella burnetii* in one-humped camels (*Camelus dromedarius*) population in vast area of NE Iran. We proved evidence that camels are an important reservoir of *Coxiella burnetii* infection in this geopolitically important part of the world.

Materials and methods

Study area

This study was conducted in three provinces: North Khorasan, South Khorasan, and Razavi Khorasan. These provinces are located at 55° 17 to 61° 15 E and 30° 24 to 38°17 N in north eastern Iran (Fig. 1). North Khorasan is a mountainous region with a cold weather in winter, Khorasan Razavi is a semi-desert region with mild weather, and South Khorasan is a semi-desert region experiencing arid conditions. The average annual rainfall is approximately 300–400 and 150 mm in the northern and southern areas, respectively. All three provinces are active in camel farming for milk and meat production (i.e., non-industrial extensively grazing camel production system is increasingly converting to a semi-industry system in the study area). One fourth of Iran's camel population is located and extensively raised in these study area (extensive grazing system of camel population, with maximal population in South Khorasan, followed by Razavi and North Khorasan provinces. Nehbandan in south province bordered with Afghanistan-Pakistan and Kanimani bordered with Turkmenistan in north province were maximal and minimal camel populated counties, respectively.

Sampling procedure

The appropriate sample size of apparently healthy camels was calculated using the following equation: $n = Z^* P_{exp}(1 - P_{exp})/d^2$

where n is the required sample size, P_{exp} is the expected prevalence, d is the desired absolute precision. $Z\propto$ is the normal deviate (1.96) at 95 % confidence level, P is the estimated prevalence, $q = 1 - P$, and d is the precision of the estimate. With P set at 0.11 and d at 0.05, a sample size of 150 was required.

From May 2012 to April 2013, 11 counties among the Khorasan provinces were randomly selected (North Khorasan, South Khorasan, and Razavi Khorasan) as a "cluster," and at least 14 camels were sampled from each cluster (Thrusfield 2007). From each camel, 20 ml blood was collected from the jugular vein; each tube was labeled with the date of collection, animal number, sex, age, and area. Samples were immediately transported to a laboratory and centrifuged (1800×*g*, 4 °C, 10 min) to obtain the serum. Sera were stored in different labeled vials at −20 °C until testing.

Serological test

Sera were tested for the presence of antibodies against *Coxiella burnetii* using a CHEKIT Q- fever ELISA kit (IDEXX Laboratories, Switzerland) according to the manufacturer's instructions. Positive and negative control sera were provided by the manufacturer. After the preparation of reagents, serum samples and controls were diluted 1/400 in microtubes using CHEKIT-Wash solution. One hundred microliter of the pre-diluted samples and controls were dispensed into appropriate wells of the 96-well plates. The 96-well plate was covered by a lid and incubated for 60 min at 37 °C; the plate was then washed three times using 300 μl washing solution. One hundred microliters of CHEKIT-Q-FEVER-Anti-ruminant- Ig-PO conjugate was dispended into each well. The microliter plate was incubated for 60 min at 37 °C in a humid chamber. Washing of the plate was repeated as described above. One hundred microliters of the substrate was dispensed into the wells. The plate was incubated at room temperature

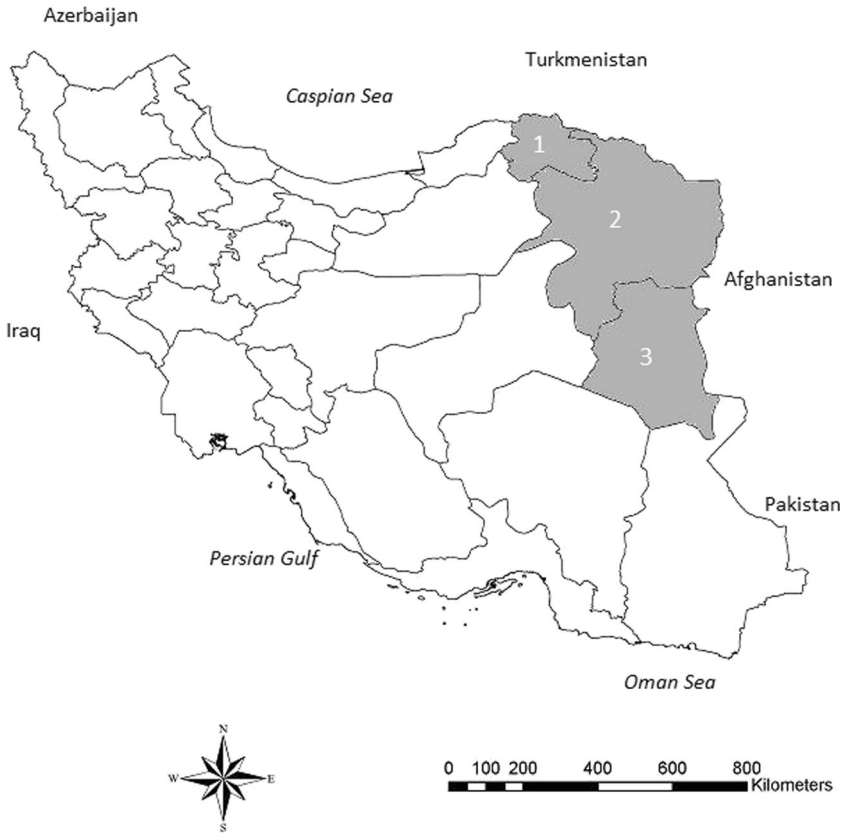

Fig. 1 Map of Iran with Khorasan (North, Razavi, and South, respectively highlighted as *1*, *2*, and *3*) provinces and the main sampling area/points in counties (see the "Materials and methods" section and Table 1). More than 25 % of Iran's camel population is located and extensively grazing camel raised in these area with high tendency toward semi-industrial camel production system. The proximity and porous long borders of the counties to Afghanistan, Pakistan, and Turkmenistan highlight the seroepidemiological consideration of the Q fever outbreak in camels, other farm animals, and humans in this geopolitically important region

for 15 min. Afterward, 100 μl of a stop solution was added to all wells and the microplates were read in an ELISA plate reader at 450 nm. Results were finally expressed as a percentage of the optical density of the test sample (%OD) calculated as below:

$\%\ OD = 100 \times (S-N)/(P-N)$, where, S, N, and P are the OD values of the test sample, negative control, and positive control, respectively.

Sera were considered to be seropositive if they had a value of ≥40, and the values of 3–40 and <30 were considered as suspect and negative, respectively. Re-analyzing suspect samples was performed as recommended by the manufacturer. The seropositivity of the camels in the study area was then used for further risk assessment analyses.

Statistical analysis

Animal level of seroprevalence and a 95 % confidence interval were calculated. Significance testing of each independent variable was performed by running a chi-square test. Univariate analyses were first conducted to identify which independent variable could be appropriate for multivariate logistic regression modeling. Predictors with $P<0.25$ were placed into a multivariate logistic regression model. A backward stepwise approach was used to identify explanatory variables, related to the seropositivity. All statistical analyses were performed using SPSS statistical software version 20 (SPSS Inc., Chicago), and a P value less than 0.05 was considered as significant.

Results

Description of seropositivity of coxiellosis in camel

Seroprevalence of *Coxiella burnetii* at animal level was 28.7 % [(95 % confidence interval (CI): 21.83, 35.56)], meaning that antibodies against *Coxiella burnetii* were detected in all selected counties. Of 11 counties, 9 counties showed camels largely infected with *Coxiella burnetii*. The proportion of seropositivity at animal level in the studied counties for camels ranged from 0 to 63.6 %; the counties with longer border and close proximity to Afghanistan were more affected (see, e.g., Nehbandan in Table 1 and Fig. 1). Antibodies against *Coxiella burnetii* were detected in 6 (21.4 %; 95 % CI: 6.21, 36.59,) out of 28 male camels and 42 (30.2; 95 % CI: 22.57, 37.83) out of 139 female camels; so, females were more affected. Descriptive insight of univariate analyses of seropositivity of coxiellosis in relation to the age, sex, district, and location for camel populations is shown in Table 1.

Table 1 Animal level prevalence of antibody to *Coxiella burnetii* with respect to sex, age, and district for camel population of wide area of Khorasan provinces, northeast Iran, bordered with Afghanistan, Pakistan, and Turkmenistan

Parameters	Tested camels (+ve)	Proportion (95 % CI)	P^a
Sex			
Female	139 (42)	30.2 (22.57, 37.83)	0.349
Male	28 (6)	21.4 (6.21, 36.59)	
Age (years)			
<3	57 (11)	19.3 (9.05, 29.55)	0.005
3–8	51 (11)	21.6 (10.31, 32.89)	
>8	59 (26)	44.1 (31.43, 56.71)	
District			
Mangali	15 (5)	33.3 (9.45, 57.15)	0.001
Cheheldokhtaran	14 (5)	35.7 (10.60, 60.80)	
Mashhad	16 (0)	0.00	
Sabzehvar	15 (3)	20 (0.00, 40.24)	
Ghochan	15 (9)	60 (35.21, 84.79)	
Robatsang	13 (3)	23.1 (0.19, 46.01)	
Boshroyeh	17 (9)	52.9 (29.17, 76.63)	
Kanimani	13 (0)	0.00	
Birjand	25 (3)	12 (0.00, 24.74)	
Sarayan	13 (4)	30.8 (5.70, 55.90)	
Nehbandan	11 (7)	63.6 (35.17, 92.03)	
Provinces			
Khorasan e Razavi	42 (10)	23.8 (10.92, 36.69)	0.365
North Khorasan	59 (15)	25.4 (14.29, 36.51)	
South Khorasan	66 (23)	34.8 (23.31, 46.29)	
Total	167 (48)	28.7 (21.83, 25.56)	

[a] Univariate analyses (χ^2 test for significancy) and $P<0.25$ were then used for multivariate analyses

Risk factors of coxiellosis in camel

Multivariate logistic regression model showed that age was correlated with the seroprevalence of coxiellosis at the individual level in camels ($P<0.05$). Based on univariate analyses, location and age were appropriate independent parameters for further multivariate logistic regression analyses. We observed that among tested risk factors influencing seropositivity of coxiellosis in camel population, age was the most remarkable risk factor. By increasing the age of camel, the chance of being infected with *Coxiella burnetii* increased (i.e., compared to age <3 years, as a reference, the risk of getting coxiellosis in camel aged 3–8 and >8 years was 1.15-fold (odds ratio (OR)= 1.15; $P=0.77$) and 3.29-fold (OR=3.29; $P=0.005$), respectively (Table 2).

Discussion

The purpose of this study was to determine the prevalence rate of *Coxiella burnetii* and associated risk factors in one-humped camels' population in NE Iran. Our novel study on the coxiellosis in widely raised one-humped camel region showed a hazardously high proportion of seropositive camels. Indeed, no previous study on the prevalence of *Coxiella burnetii* existed in this region. The results of the present study provide a useful insight into the prevalence and distribution of *C. Burnetii* infection in one-humped camel population in northeast Iran. About 29 % of camels were seropositive. The prevalence of seropositive camels per district varied with significant difference in prevalence between the different districts ($P=0.001$). This may be partly related to differences in management, hygienic measures, and proximity to Afghanistan (i.e., the counties closer to the border with Afghanistan-

Table 2 Multivariate logistic regression model showing factors influencing the risk of coxiellosis in camel population in wide area of northeast Iran

Independent variable	β (SE)	OR (95 % CI)	*P*
Age, years (*n*)			
<3 (57)	1, reference	–	–
3–8 (51)	0.14 (0.48)	1.15 (0.45, 2.93)	0.77
>8 (59)	1.19 (0.43)	3.29 (1.43, 7.59)	0.005

By increasing the age of camel, the chance of being infected with *C. brunette* increases (i.e., the risk of getting coxiellosis in camel aged 3–8 and above 8 years could be 1.15- and 3.29-fold, respectively, higher than those under 3 years old, as reference). Age <3 was used as reference

OR odds ratio, *CI* confidence interval

Pakistan showed higher seropositivity to Q fever). Geopolitically, the study zone is important, worldwide, owing to the presence of international peacekeeper/NATO forces, and ordinary people are in close contact with camel and thus each other. Since our sampling was mainly performed in rural areas, poor hygiene could exacerbate the spread of *Coxiella burnetii* (Luoto 1960; Lyytikäinen et al. 1998).

It appears that the camel is likely to harbor high levels of *Coxiella burnetii* and capable of shedding the bacterium through milk, blood, feces, and urine and especially in birth by-products (Arricau-Bouvery and Rodolakis 2005; Tissot-Dupont et al. 2007; Schimmer et al. 2010; Bielawska-Drózd et al. 2013; Tozer et al. 2013; Mohammed et al. 2014; Schimmer et al. 2014; O'Connor et al. 2015; Pimenta et al. 2015; Moffatt et al. 2015; Nusinovici et al. 2015; van den Brom et al. 2015). Environment contamination of *Coxiella burnetii* events lasts for months and even years; so, inhalation of dust (aerosol contamination) is also very critical for farm animals and public health. Also, different climates and various densities of camels might be related to the spread of *Coxiella burnetii* and thus seropositivity. Nehbandan, which showed high seroprevalence of coxiellosis for camels, is a district with high density of camels and dry/windy area, affecting in urban area.

In camel population, seroprevalence increased by age, and the logistic regression model showed that camels older than 8 years are most likely to be seropositive than those less than 8 years old (P=0.005). This finding is in agreement with others (McCaughey et al. 2010; Ruiz-Fons et al. 2010), suggesting higher exposure of older camels to *Coxiella burnetii*.

In NE Iran, the animal studies show wide variation in seroprevalence, with sheep having higher average seroprevalence (36.5 %) compared to goat (29.8 %) or commercial dairy cow (22.3 %) (Keyvani Rad et al. 2014). Despite of much lower number/density of camels in the study area, camel Q fever seropositivity was similar to what we observed in sheep/goats in the region (Keyvani Rad et al. 2014). This can be epidemiologically noticeable. This together with current studies imply that *Coxiella burnetii* exists and is circulating among farm animals that are the most common source of Q fever outbreak in humans living in rural and urban areas. The results reported herein showed that *Coxiella burnetii* is endemic and hazardously distributed in the study area, which can lead to rural and urban Q fever outbreak in animals and human.

Except signs of mild and undetermined previous history of abortion, no specific clinical signs were recorded during sampling in any of the seropositive camels. Many other authors previously have reported antibodies against *Coxiella burnetii* in the sera of camels (Mathur and Bhargava 1979), with no clinical sign. It is generally known that farm animals serve primarily as asymptomatic carriers of *Coxiella burnetii* and that organism resides abundantly in their placenta and udder and may be shed for extended periods in milk, urine, feces, and birth fluids thus exposing other animals, and people to *Coxiella burnetii* (Maurin and Raoult 1999; Tissot-Dupont et al. 2007; Tozer et al. 2013; O'Connor et al. 2015; Pimenta et al. 2015). Therefore, owing to camel booming potential for meat/milk production, biomedicine, and agrotourism in this harshly dried environment (i.e., the agroecologically and geopolitically important current study zone), ordinary people are simply contacted by camel, and camel contribution to the risk of Q fever in human should be established without reasonable doubt.

In short, the prevalence of *Coxiella burnetii* in one-humped camels' population in NE Iran is widely spread, and camelids might have an important role in the epidemiology of Q fever among human population in Iran and neighboring countries. Implementation of strict hygienic measures in farm where camels are kept is important to reduce *Coxiella burnetii* contamination. In particular, attention should be given to hygienic disposal of placentae and dead or aborted fetuses, preferably by incineration, as well as prompt removal and replacement of bedding materials with birthing fluid. Workers and farmers should also be advised on measures to protect themselves while handling camels, such as wearing face masks, gloves, and overalls.

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Conflict of interest We declare that we have no conflict of interest.

Ethical standards The manuscript does not contain clinical studies or patient data.

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