Seroprevalence of Q fever in farm animals in Saudi Arabia.

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Abstract

This study was undertaken to determine the seroprevalence of Q fever in domestic livestock in Saudi Arabia. Serum samples from 489 camels, 428 cattle, 630 sheep and 423 goats, of either sex, from different localities were tested for antibodies against *C. burnetii* using a Q fever indirect enzyme-linked immunosorbent assay (ELISA). A subsample of 307 animals of different species was simultaneously tested for *C. burnetii* antibodies by ELISA and indirect immunofluorescence (IFA). The overall seroprevalence was 30.71%. Prevalence by species was 51.53%, 30.67%, 34.04% and 12.38% in camels, cattle, goats and sheep, respectively. Significant differences in seroprevalence were recorded between species and locations. The prevalence was overtly higher in adult as compared to young animals. No significant difference was recorded between male and female animals. There was a close agreement between ELISA and IFA results in cattle and camels while the results of the two tests were at variance in sheep and goats. The results indicate that the domestic livestock and the camel are the source of Q fever endemicity in Saudi Arabia.

Keywords: Q fever, ELISA, Farm animals, IFA, Saudi Arabia, Seroprevalence.

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Introduction

Q fever (Coxiellosis) is a ubiquitous anthropozoonosis caused by *Coxiella burnetii*, an intracellular bacterium with a wide range of vertebrate and invertebrate hosts including man, domestic animals and birds [1]. The organism has been reported from all parts of the world, with the exception of the Antarctic region [2]. In nature, *C. burnetii* circulates between ticks and small wild mammals [3]. In humans and domestic animals, the infection is usually aero-genic, but could also be acquired by ingestion and other routes. *C. burnetii* is characterized by extremely high infectivity and resistance in the environment [4].

Following a long period of underestimation and misdiagnosis or undiagnosed [5,6], Q fever has recently re-emerged as a public health and veterinary problem in many countries. A major epidemic of Q fever affecting nearly 4,000 people has been reported during 2007-2010 in the Netherlands, in which

infected dairy goats were identified as the most likely source of infection [7,8]. A multistate outbreak of human Q fever has also been reported recently in the USA [9].

The earliest reference to Q fever in Saudi Arabia dates to the 1960's when the disease was first recognized as holo-endemic among the inhabitants of that country [10,11]. Over the next 50 years, three reports on human Q fever and two on Q fever in animals have been published. In humans, a single case of acute Q fever leading to meningoencephalitis was reported in a US soldier returning from Saudi Arabia after the first Gulf war [12], while asymptomatic infection was reported in four other soldiers, presumably after exposure to animals in that country [13]. More recently, 18 out of 51 persons in Saudi Arabia tested by immunofluorescence were positive to Q fever antibodies, but no information was available on their history or location [14]. A similarly striking paucity of information applies to Q fever in animals in Saudi Arabia, where only two reports are found in the literature, one on serological detection

of *C. burnetii* in Saudi camels [15] and the other on seroprevalence in some wild desert ungulates [16]. No information on the prevalence of Q fever in other agroeconomically important animal species in Saudi Arabia is found

The present study was therefore, undertaken to investigate the seroprevalence of *C. burnetii* (Q fever) in different species of farm animals in Saudi Arabia. This is considered important since farm animals infected with *C. burnetii* sometimes experience reproductive disorders, besides being the main source of human infection [2].

Materials and Methods

Animals

During 2012 and 2013, a total of 1970 serum samples from animals, comprising 428 cattle, 630 sheep, 423 goats and 489 camels of either sex were investigated for seroprevalence of antibodies against C. burnetii. The animals were selected randomly at farms, markets, slaughter-houses and free ranged herds in different locations. They were all divided into young (sexually premature) and adult (sexually mature) animals according to the following criterion: in bovines; young is less than 2 years and adult is equal to or more than three years - in sheep/goats young is less than 1 year and adult equal or more than 1 year and in camels young is less than 4 years and adult equal or more than 4 years. Camels belonged to Maghater, Majahim and mixed breeds; sheep belonged to Najdi, Naimi and Harri breeds and goats belonged to Ardi and Demasqi breeds. All cattle were locally born and bred Friesian-Holstein. The systems under which animals are kept are either intensive or extensive (housed). Animals held under intensive rearing system are those which are under the shade and food was provided in the same place while those under extensive system are kept in a paddock and they are allowed free ranging for few hours every day (free-ranged).

All animals were clinically normal at the time of sampling, except some camels that harboured ticks. None of the adult female animals included in this study was pregnant while some females were lactating. Also, none of the sampled adult females had a history of abortion or stillbirth although many of them belonged to herds where abortions of unknown etiology have been previously recorded in some herd members.

Serological tests

Ten ml blood samples were collected by jugular venipuncture from each animal into plain vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, N.J., USA). The samples were allowed to clot at room temperature for 3 h and serum was separated by centrifugation at 1,500 g for 15 min and stored at -20°C. The sera were tested for antibodies against *C. burnetii* using an indirect enzyme-linked immunosorbent assay (ELISA) designed to detect antibodies against purified phase I and II *C. burnetii* antigens (CHEKIT-Q fever enzyme immunoassay; IDEXX laboratories, Bommeli Diagnostics,

AG, Bern, Switzerland). A monoclonal anti-ruminant IgG conjugated with horseradish peroxidase (HRP) was used to detect positive bovine, ovine and caprine sera. A peroxidase-conjugated goat anti-camel IgG (Triple J. Farms, 777 Jorgensen Place, Bellingham, WA 98226, USA) was used for detecting seropositivity in camels. The tests were performed according to manufacturer's protocol in microtiter plates precoated with inactivated *C. burnetii* antigen (Nine Miles reference strain). Known positive and negative control sera were run in each test plate. The optical density (OD), corresponding to the degree of color change, and hence to the concentration of antibodies, was determined at 450 nm using a microtiter plate reader. The optical density percentage (OD%) of the samples was determined as follows:

OD% of the test sample = 100 (S - N)/(P - N)

Where S, N and P are the OD values of the test, negative control and positive control sera, respectively. A good visual cut-off was observed at OD% equals 40; therefore, samples were considered positive only if their OD percentage value is \geq 40.

For additional confirmation, serum sub-samples from 92 camels, 72 cattle, 72 sheep and 71 goats were simultaneously tested by ELISA and indirect immunofluorescence assay (IFA). IFA test was designed to detect IgG antibodies against phase II C. burnetii (Nine Miles strain) antigen in serum samples (Vircell, S. L. Pza. Dominguez Ortiz I. Poligono Industrial Dose de Octubre. 18320 Santa Fe, Granada, Spain). Each serum sample was diluted at 1:64 as the first positive dilution, and serial twofold dilutions were made to titrate positive samples up to a maximum titer of 1:8192. Specific fluorescein isothiocvanate (FITC)-conjugated anti-species immunoglobulins were used, comprising FITC conjugated rabbit anti-bovine, anti-sheep and anti-goat IgGs (Gentex Inc., 2456 Alton Pkwy, Irvine, CA 92606, USA) and FITC conjugated goat anti-camel IgG (Triple J. Farms, 777 Jorgensen Place, Bellingham, WA 98226, USA). The tests were performed following manufacturer's recommendations and the results were determined using a fluorescence microscope (Axioskop 2 plus; Zeiss, Gttingen, Germany) under 400× magnification. Samples showing bright apple green fluorescence of coco-bacillary morphology against a dark background at dilutions >1:64 were considered positive.

Statistical analysis

Data were analysed with the incidence of C. burnetii coded as a binary dependant variable (0 for sero-negative and 1 for seropositive animals). Frequencies and means of C. burnetii prevalence and ELISA titres were computed using Statistical Analysis System V. 9.1 software for Windows. A probability value of $p \leq 0.05$ was considered statistically significant. Logistic models were utilized to examine the associations of independent variables with the incidence of Q-fever. The general formula of the model was:

Logit $P(x) = \beta_0 + \beta_i(x_i)$

where β_0 , β_i are regression coefficients and xi the effects of independent variables of species, breed, sex, age, rearing method and sampling location. Odd ratios (OR) were estimated from the logistic models, and calculated as the rate of odds for x=1 to the odds of x=0. The relationship between ELISA and IFA tests was performed using the PROC CORR procedure.

Results

ELISA test

Serum samples from a total of 605 animals were positive for specific IgG antibodies to *C. burnetii*, giving an overall seroprevalence of 30.71% (Table 1). The mean ELISA OD percentage was 103.03% for positive samples. The highest mean OD percentage was recorded in cattle, followed in descending order by camels, goats and sheep.

Table 1. Seroprevalence and titration (OD%) results of Q fever in farm animals in Saudi Arabia.

		Results	No.	%	Titration (OD%)		
		results	140.	70	Mean	Min	Max
Species	Cattle	-ve	297	69.39	8.35	0	39.18
		+ve	131	30.61	126.49	41.33	228.02
	Camels	-ve	237	48.47	14.60	0	38.97
		+ve	252	51.53	113.37	40.47	375.13
	Sheep	-ve	552	87.62	10.48	0	39.65
		+ve	78	12.38	60.66	40.07	184.00
	Goats	-ve	279	65.96	8.97	0	39.54
		+ve	144	34.04	99.38	40.11	226.19
Age	Adult	-ve	994	65.92	12.02	0	39.65
		+ve	514	34.08	106.54	40.04	375.13
	Young	-ve	371	80.30	6.25	0	39.26
		+ve	91	19.70	103.51	42.42	226.19
Gender	Male	-ve	244	85.92	6.46	0	38.97
		+ve	40	14.08	66.62	40.90	202.08
	Female	-ve	1121	66.49	11.32	0	39.90
		+ve	565	33.51	108.91	40.07	375.13
Rearing system	Housed	-ve	982	79.26	10.08	0	39.65
		+ve	257	20.74	101.15	40.62	237.13
	Free- Ranged	-ve	383	52.39	11.37	0	39.54
		+ve	348	47.61	109.74	40.07	375.13

All cattle were housed; other species were either housed or free-ranged.

Camels showed the highest proportion of Q fever seropositivity, with an overall prevalence of 51.53%. The second highest seroprevalence was recorded in goats (34.04%),

followed by cattle (30.61%) and the least in sheep (12.38%). The seroprevalence of *C. burnetii* varied in different geographical locations, with highest prevalence in Harad (69.6%) and lowest in Riyadh (13.7%) in Central Saudi Arabia.

Logistic regression analysis and estimation of odd ratios (OR) of the results indicated highly significant differences in seroprevalence of Q fever depending on the variable (Table 2). The odds of being positive for Q fever antibodies in adults were found to be 2.11 times larger than for young animals. Similarly, highly significant differences in the risk of being seropositive for Q fever were found between different species, animal location, and whether the animals were housed or reared free-range. Options for analysing interactions between variables in the model were not investigated.

Table 2. Q fever in animals; logistic regression analysis.

Source	P-value	Odds ratio	95% CI for Odds ratio
Intercept	<.0001		
Species			
Cattle	0.9223	2.57	1.27-5.21
Camel	<.0001	6.98	4.59-10.60
Goat	0.8175	2.72	1.93-3.82
Sheep	Reference		
Age			
Adult	<.0001	2.11	1.50-2.94
young	Reference		
Location			
Jouf	0.6330	0.99	0.54-1.82
Kharj	0.0230	2.07	0.96-4.43
Riyadh	0.0100	0.77	0.47-1.27
Tabrak	Reference		
Rearing			
Extensive	0.1540	1.69	0.82-3.49
Intensive	Reference		

Immunofluorescence test

ELISA and IFA results are given in Table 3. Statistical analysis showed highly significant differences in seroprevalence of Q fever in different species using both serological tests. There was an excellent level of agreement between ELISA and IFA results in cattle and camels. By contrast, the level of agreement between the two tests in sheep and goats was poor (Table 3). Means and ranges of ELISA and IFA antibody titers showed that the highest mean ELISA OD percentage was recorded in camels, followed, in descending order, by cows, goats and sheep, while the highest mean IFA titer was recorded in cows, followed by camels, sheep and goats. High IFA titers were

recorded in some animals, up to 1:8192 or more in camels and cattle and up to 1:4096 in sheep and goats (Table 3). The range of IFA antibody titer for cattle and camel was $64- \geq 8192$ whereas it ranged between 64 and 4096 in both goats and sheep.

Table 3. Comparison between ELISA and IFA assays for C. burnetii antibodies in animals. ELISA (OD%) and IFA Anti-C. burnetii antibody titers in seropositive animals.

Species	N	Agreement between ELISA and IFA (%)	IFA		
			Mean Titer + SE	Range	
Cattle	72	51.4%	1785.34+328.53	64 - >8192	
Camels	92	62%	793.82+213.60	64 - >8192	
Goats	71	32%	735.27+158.47	64 - 4096	
Sheep	72	37.5%	787.09+134.36	64 - 4096	

Discussion

The screening and diagnosis of Q fever in animals relies mainly upon serology [2], and the methods most commonly used are the indirect ELISA and IFA assays. The former method has 92–95% sensitivity and 100% specificity relative to indirect IFA test [17-20]. It is also economically feasible and easy to perform; therefore, it offers a convenient method for large-scale screening of Q fever antibodies in animals [21]. Ready-to-use ELISA kits are available for determining the prevalence of Q fever in animals. In the present study, we used the indirect ELISA CHEKIT Q fever test to screen antibodies to *C. burnetii* among indigenous livestock in Saudi Arabia. The same test has been widely used for Q fever surveys in various species of ruminant farm animals and wild ungulates [16], as well as kangaroos [22] and even humans [23].

Although O fever in man and animals has been widely reported world-wide [2,3], there is a dearth of information regarding its prevalence in Saudi Arabia. The present study is the first documentation of this disease in cattle, sheep and goats and the second in camels in the Kingdom. The results indicate that Q fever is common in all species of indigenous farm animals, with an apparent overall seroprevalence exceeding 30%. The prevalence, however, varies between different species and for the same species in different localities. This could be partly attributed to differences in management and prevailing climatic conditions in different areas. In the present study, an exceptionally high seroprevalence (>51%) was recorded in camels. In a previous study by Hussein and others [15] an even higher prevalence (62%) was observed in Saudi camels. Similarly, high prevalence (66%) were recorded in camels in Egypt [24], Chad (80%) [25] and also in nomadic camels (100%) in eastern Ethiopia [26]. This exceptionally high prevalence could be attributed to poor hygienic and management conditions under which camels are kept, as well as their heavy exposure to contaminated dust and sand during sandstorms in desert areas. These conditions, coupled with the wide spread tradition of consuming raw camel milk,

underscore the main role that camels might play in the transmission of Q fever to humans in this part of the world, a role that probably extends beyond the Arabian Peninsula into other parts of Asia and north Africa where Q fever has also been commonly reported in camels [27,28]. The relatively high prevalence of antibodies to *C. burnetii* in other species of farm animals in the Kingdom could also be associated with generally poor management practices and exposure to sandstorms, especially during the early part of the summer.

A significantly higher serological prevalence of Q fever was recorded in adult compared to young animals. This agrees with previous observations in man and animals [15,29-32]. It should be pointed out, however, that the infection can occur at any age. In the present study, Q fever antibodies were found in a one-day old lamb, which could have been infected in utero. On the other hand, no significant intersex difference was recorded in the present animals, which also agrees with other investigators [4,29,33].

The absence of overt clinical signs in Q fever positive animals, despite high antibody titers in some individuals, was not unexpected since this infection in animals is usually asymptomatic [4]. However, the possibility that clinical signs of Q-fever sometimes occur cannot be overlooked, especially if the animals are subjected to stressful conditions such as advanced pregnancy. In these cases, abortion, stillbirth, retention of the placenta, placentitis, endometritis and other manifestations might be observed [34-37].

There was a close agreement in the prevalence values of Q fever in cattle and camels, as determined by ELISA or IFA, indicating that either of these assays can be used for screening Q fever or for confirming one another. On the other hand, the discrepancy between ELISA and IFA results in sheep and goats is difficult to explain at present and further studies are needed to compare sensitivity, specificity and predictive values of these tests in these animals. A similar finding was observed in goats by Rousset and others [38] as 14 of the samples which gave negative ELISA results and positive IFA results. Another study by Emery and others [39] also observed the same in sheep, goat and cattle using ELISA and CFT. In both studies they attributed the discrepancies to the differences in the C. burnetii antigens used in commercial kits. Furthermore, they concluded that the typical serological profiles of Q fever in ruminants are largely unknown and the tests available are based on the Phase II antigen or a mix of antigen Phases but are unable to discriminate between the different antigen antibodies. Kennerman and others [30] reported a significant relationship between the age and the seroprevalence of Q fever in sheep in Turkey. They indicated that, in infected flocks, seroprevalence in older sheep was significantly higher than in younger sheep.

Finally, the high seroprevalence of Q fever in indigenous farm animals in Saudi Arabia indicates that the Kingdom is an endemic focus of Q fever. In the light of the potential role of the Q fever agent as a cause of reproductive disorders in animals, in addition to its zoonotic nature and importance from the public health standpoint, concerted efforts should be

undertaken to reduce its prevalence in animals and to protect workers at higher risk of being exposed to infection.

The overall seroprevalence of Q fever in Saudi Arabian farm animals is 30.71%. This relatively high prevalence indicates that Saudi Arabia is an important endemic focus of Q fever. A close agreement between ELISA and IFA results is recorded in camels and cattle but not sheep and goats. The prevalence of Q fever in camels exceeds 51% reflecting the poor management and hygienic conditions under which these animals are kept and their frequent exposure to contaminated dust and sand during sandstorms in the desert. The high prevalence of Q fever in camels, coupled with the widespread habit of consuming raw camel milk, underscore the key role of camels in the transmission of human Q fever in Saudi Arabia. Efforts should, therefore, be made to reduce the prevalence of Q fever in the Kingdom's animals using different control measures, including introduction of animal vaccination, and to protect workers at higher risk of exposure to infection.

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