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## Prevalence of Antibodies to *Coxiella burnetii* in Camel Milk in Riyadh Region, Saudi Arabia: a Comparison with Serum

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### ABSTRACT

Antibodies against *Coxiella burnetii*, the causative agent of Q-fever, were detected in lactating camels, cows and goats in Riyadh region, Saudi Arabia, using an indirect ELISA test. A total of 246 milk samples collected from 69 camels, 90 cows and 87 goats were tested. Milk samples from 43 camels (62.32%), 30 cows (33.33%) and 22 goats (25.29%) were positive for anti-*C. burnetii* antibodies. Serum samples collected simultaneously from the same animals and tested by ELISA revealed anti-*C. burnetii* antibodies in 46 camels (66.67%), 38 cows (42.22) and 14 goats (16.20%). A significant correlation between ELISA results in milk and serum was observed in the species tested. These results confirm that ELISA can be used in milk instead of serum to detect antibodies against *C. burnetii* in lactating camels and other animals.

**Keywords:** ELISA . *Coxiella burnetii* . Antibodies . Livestock . Saudi Arabia

Q-fever is an important zoonosis caused by *Coxiella burnetii*, a ubiquitous intracellular bacterium which infects man and a large number of different animal species (Angelakis and Raoult 2010). Farm animals are the primary source of infection to humans and most of the infected people are working in close contact with animals (Anderson *et al.*, 2013). Animals infected with *C. burnetii* are usually asymptomatic. However, abortion, mastitis, infertility and other reproductive disorders may sometimes occur in animals, leading to significant economic losses. Definitive diagnosis of the infection is best done using molecular methods such as PCR (Mohammed *et al.*, 2014); however, these methods are expensive, laborious and are not readily available in many laboratories. Hence, diagnosis and screening of coxiellosis in animals is still largely based on serological assays such as enzyme-linked immunosorbent (ELISA) and indirect immunofluorescence (IFA) assays (Guatteo *et al.*, 2007;

Angelakis and Raoult, 2010). Using these methods, we recorded a high prevalence of anti-*C. burnetii* antibodies in the sera of indigenous livestock in Saudi Arabia, with highest prevalence in camels (Gar El Nabi, 2014). One of the problems encountered has been the strong resentment by Saudi camel owner to blood sampling of their animals, particularly if they are lactating. Several studies have shown that ELISA milk test for the screening of Q-fever antibodies in lactating cows, ewes and goats offers a cheaper and more acceptable alternative to ELISA serum tests (Paiba *et al.*, 1999; Guatteo *et al.*, 2007; Garcea-Pérez *et al.*, 2009; Agger *et al.*, 2010; Ryan *et al.*, 2010; Khalili *et al.*, 2011; Gyuranecz *et al.*, 2011). Comparing the performance of ELISA Q-fever test in matched milk/serum samples of 448 lactating cows, Guatteo *et al.* (2007) recorded a very good level of agreement between the results obtained in milk and serum. The present study was undertaken to compare the use of indirect ELISA for

the screening of *anti-C. burnetii* antibodies in milk versus serum from lactating camels in Saudi Arabia. Samples from dairy cows and goats were included for comparison. To our knowledge, this study represents the first study from anywhere that compares the prevalence of *C. burnetii* antibodies in camel's milk versus camel's serum using ELISA. It is also the first study in Saudi Arabia comparing anti-*C. burnetii* antibody levels in milk versus serum in cows and goats.

## MATERIALS AND METHODS

### Animals

Milk and serum samples were collected from a total of 246 randomly selected lactating animals, comprising 69 camels, 90 cows and 87 goats. The camel and goat samples were collected from naturally grazing animals in different parts around Riyadh region whereas the bovine samples were collected from a dairy farm in Al-Kharj, about 75 km south of Riyadh town. Ages of these animals ranged between 6-10 years in camels, 2-3 years in cows and 1.5-3 years in goats. No information was available on the number of parturitions for the sampled animals, as all of them, with the exception of cows, were naturally grazing animals reared by traditional methods, and no production records were kept. However, as we understood from the herders, most of the animals were in the second lactation season. All the animals were clinically normal at the time of sampling and none of them was vaccinated against Q fever.

### Sampling procedures

Prior to milk sampling, the udders and teats were thoroughly washed and dried, and the first few streams of milk were discarded. 40 mL milk samples were collected from each animal into clean, sterile containers. The samples were kept in ice during transit from the field to the laboratory. Upon arrival to the laboratory, the samples were immediately centrifuged at 1000 x g for 10 min, and the fat fraction was discarded, while the defatted fraction was frozen at -20°C until tested. Each sample was diluted 1:5 in phosphate buffered saline (PBS) solution. For serum collection, 10 mL jugular blood samples were drawn from each animal into a plain, evacuated tube and allowed to clot at room temperature for 3 h. Serum was separated

from the clotted blood samples by centrifugation at 1,500 g for 15 min and stored at -20°C. For testing, the serum samples were diluted 1:100 in PBS.

### ELISA Tests

ELISA assays for the presence of antibodies to *C. burnetii* were performed using the CHEKKIT-Q fever enzyme immunoassay designed to detect antibodies against phases I and II *C. burnetii* antigens (IDEXX laboratories, Bommeli Diagnostics, AG, Bern, Switzerland). A horseradish peroxidase (HRP)-conjugated goat anti-camel IgG (Triple J. Farms, 777 Jorgensen Place, Bellingham, WA 98226, USA) was used in the case of camels, whereas a monoclonal anti-ruminant HRP-conjugated IgG supplied with the kit was used to test samples of cattle and goats. The anti-camel IgG conjugate used in the present study was previously used to compare ELISA with IFAT tests for the detection of anti-*C. burnetii* antibodies in camels sera and the results closely agreed with each other (Kappa=86%) (Hussein et al., 2014).

ELISA tests were performed in 96-well micro-titer plates pre-coated with inactivated *C. burnetii* antigens (Nine Miles reference strain). Each test was performed in duplicate, with positive and negative control samples being included in each plate. Control sera for the camels were designated as positive or negative on the bases of three tests in our laboratory: ELISA, indirect fluorescent antibody test and PCR. Control sera provided with the kit were used for testing cows and goats. Tetramethylbenzidine was used as the enzyme substrate and the coloring reaction was stopped after incubation for 10 min at 37°C using 05M H<sub>2</sub>SO<sub>4</sub>. Both the enzyme substrate and stopping solution were supplied with the kit. The optical density (OD) was determined at 450 nm in a micro-titer plate reader and the sample/positive percentage (S/P %), which corresponds to the intensity of color change and hence antibody concentration was calculated as follows:

$$\text{S/P \% of the test sample} = 100 \left( \frac{S_{OD} - N_{OD}}{P_{OD} - N_{OD}} \right)$$

where S<sub>OD</sub>, N<sub>OD</sub> and P<sub>OD</sub> are the OD values of the tested, negative control and positive control sera, respectively. As recommended by the manufacturer, samples with S/P% values > 40 were considered positive.

Serum samples from the same animals were simultaneously tested in the same manner by ELISA for the presence of specific antibodies against *C. burnetii*.

**Table 1.** Results of ELISA test for Q fever antibodies in milk and serum

Species	No.	S/P %					
		Positive	%	Mean	+SD	Min	Max
Cows	90	30	33.33	83.19	5.365	46.05	164.65
Camels	69	43	62.32	143.20	13.55	40.84	284.51
Goat	87	22	25.29	75.37	6.30	41.94	168.15

  

ELISA Detection of Q fever Antibodies in Serum						
Species	No.	Positive	%	Mean	+SD	Min
Cows	90	38	42.22	120.30	5.85	42.02
Camels	69	46	66.67	186.65	19.24	40.84
Goats	87	14	16.10	60.89	3.72	42.50

**Table 2:** Spearman correlation for ELISA results in milk and serum

Species	Correlation Coefficient	Probability
Camels	0.65	<0.0001
Cows	0.38	0.001
Goats	0.41	0.002

### Statistical analysis

Spearman correlation analysis was used to determine the relationship between the prevalence of anti-*C. burnetii* antibodies in milk and serum. The data were analyzed with the incidence of *C. burnetii* coded as a binary dependent variable (0 for sero-negative and 1 for seropositive animals). Frequencies and means of *C. burnetii* prevalence were computed using Statistical Analysis System V. 9.1 software for Windows (SAS 2009). A probability value of  $p \leq 0.05$  was considered statistically significant.

### RESULTS

Out of the 246 defatted milk samples collected from camels, cows and goats and tested by ELISA, a total of 95 samples (38.62%) were positive for anti-*C. burnetii* antibodies. Simultaneous ELISA testing of serum samples from the same animals revealed 98 (39.84%) sero-positive

samples (Table 1). All camels, cows and goats that were positive for anti-*C. burnetii* antibodies in milk were also positive in serum, whereas 3 camels and 8 cows were positive only in serum and negative in milk. In the case of goats, 14 animals were positive for anti-*C. burnetii* antibodies both in serum and milk and 8 additional animals were positive only in milk. The highest antibody prevalence was recorded in camels, followed by cows then goats. Correlation coefficient and p-values for each species (Table 2) indicated a highly significant relationship between the prevalence of antibodies against *C. burnetii* in milk and serum in camels and other species.

### DISCUSSION

The present study indicates that ELISA test can be used reliably to detect antibodies against *C. burnetii* in milk instead of serum in lactating camels in Saudi Arabia. Because of its non-invasive nature, milk sampling is less likely to be resented by Saudi animal owners. It is also cost-effective, easier to perform and less likely to be subject to environmental contamination compared with other animal secretions such as urine, faeces and vaginal secretions (Roest *et al.*, 2013). Also with ELISA milk testing available, the collection of blood for serological screening of Q fever will not be required for lactating animals.

There are no previous studies on the use of ELISA tests for the detection of antibodies against *C. burnetii* in camel's milk. However, several authors have previously investigated the use of ELISA tests on bulk-tank milk (BTM) samples as a means of determining the prevalence of *C. burnetii* in national dairy cattle herds. Ryan *et al.* (2011) reported a prevalence of 37.9% of BTM samples collected from 290 dairy herds in Ireland, while Paiba *et al.* (1999) reported antibodies in 21% of the samples collected from dairy herds in the United Kingdom. Also on the basis of ELISA testing of BTM samples for *C. burnetii* antibodies, a prevalence rate of 45.5% was recorded in dairy herds in Eastern Iran (Khalili *et al.*, 2011), and 40.7–55.5% in Portugal (Anastácio *et al.* 2012). In Denmark, Angen *et al.* (2011) investigated the prevalence of coxiellosis using a combination of ELISA and PCR in 1,514 milk samples from 12 dairy herds and reported antibodies in 25% and *C. burnetii* DNA in 37.8% of the samples using ELISA and PCR, respectively. Ruiz-Fons *et al.* (2011) studied

the prevalence of *C. burnetii* in dairy sheep in Spain and concluded that testing BTM by ELISA is a useful index of the seroprevalence of coxiellosis in dairy sheep. Guatteo *et al.* (2007) compared the prevalence of *C. burnetii* antibodies in dairy herds by applying ELISA tests in both milk and serum. These authors tested matched milk and serum samples from 448 lactating cows in France and reported anti-*C. burnetii* antibodies in 264 (58.9%) serum and 257 (57.37%) milk samples, giving a very good level of agreement between the results in milk and serum. The present results are concordant with Guatteo *et al.* (2007) in that a significant agreement existed between ELISA results for *C. burnetii* antibodies in milk and serum. It appears, therefore, that ELISA tests based on milk samples provide a convenient tool for assessing the status of *C. burnetii* in lactating animals on national levels.

In the present study, The number of camels and cows positive for antibodies against *C. burnetii* tended to be somewhat higher in serum as compared to milk while in goats a higher positivity was recorded in milk, probably indicating higher sensitivity of the milk test than serum test in that species. The present study also showed that the highest prevalence of antibodies against *C. burnetii* in serum and milk was recorded in camels (66.76% and 62.32%, respectively). This was not unexpected since similar or even higher prevalence of antibodies against coxiellosis in camels has also been reported in other camel rearing areas e.g., 66% in Egypt (Soliman *et al.*, 1992), 80% in Chad (Schelling *et al.*, 2003) and up to 100% in Eastern Ethiopia (Gumi *et al.*, 2012) indicating the high susceptibility of camels to *C. burnetii*. Despite this high serological prevalence, however, camels resemble other species of farm animals in that the infection is asymptomatic.

In camel-rearing countries like Saudi Arabia, it is particularly important to carry out a nationwide investigation into the prevalence of anti-*C. burnetii* antibodies in camels' milk, not only because of the very high serological prevalence of coxiellosis in these animals but also because of the widespread tradition of consuming raw camel milk among the natives of those countries (Schelling *et al.*, 2003; Hussein *et al.*, 2008; Gumi *et al.*, 2012). Studies are also needed to assess the potential role of *C. burnetii* as a cause of abortion and infertility in camels.

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## CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

## ETHICAL STATEMENT

We confirm that our research has been carried out according to the ethical guidelines of King Saud University, and that international guidelines for animal welfare were followed

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