

# Serological prevalence of *Coxiella burnetii* in captive wild ruminants in Saudi Arabia

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**Abstract** Serum samples from sand gazelles ( $n=227$ ), mountain gazelles ( $n=232$ ), and Arabian oryx ( $n=96$ ) reared in captivity in Riyadh, Saudi Arabia were tested for the presence of *Coxiella burnetii* antibodies using an indirect enzyme immunoassay. *C. burnetii* antibodies were present in 18.3%, 7.3%, and 46.9% of these animals, respectively. The difference in serological prevalence between the three species was statistically significant. Age- and sex-related differences in prevalence were also observed. This study is the first record of *C. burnetii* antibodies in Arabian gazelles.

**Keywords** Arabian oryx (*Oryx leucoryx*) · Sand gazelle (*Gazella subgutturosa marica*) · Mountain gazelle (*Gazella gazella*) · *Coxiella burnetii* · ELISA · Saudi Arabia

## Introduction

Q fever (Coxiellosis) is an anthroponosis of worldwide distribution caused by *Coxiella burnetii*, an obligate intracellular bacterium that infects a wide range of vertebrate and invertebrate hosts. The organism is characterized by extremely high infectivity and tenacity in the environment (Maurin and Raoult 1999). In nature, it

circulates primarily among ticks and small mammals and birds, whereas in humans, the vast majority of cases are acquired as aerosol infections from contact with infected animals or their birthing fluids and fetal membranes (Maurin and Raoult 1999; Marrie and Raoult 2005; Kazar 2005; Arricau-Bouvery and Rodolakis 2005). Recent reports from the Netherlands described a large human epidemic of Q fever which affected nearly 2,300 people; the source of infection could not be determined with certainty, but dairy goats were blamed as the most likely source of infection to humans (Van den Borm and Vellema 2009; Schimmer et al. 2009; CDC 2010).

Although Q fever has been recognized as a public health problem in Saudi Arabia since the 1960s (Gelpi 1966; Lippe et al. 1968), surprisingly, little has been published concerning its prevalence and epidemiology in man and animals in that country. A recent survey of indigenous Saudi camels, however, detected *C. burnetii* antibodies in 285 (62%) out of 460 animals (Hussein et al. 2008). This high prevalence in camels, coupled with the long-standing tradition of consuming raw camel milk, may be partly responsible for the high endemicity of coxiellosis among Saudis (Lippe et al. 1968). Camels were also suspected as the probable source of acute Q fever, leading to meningo-encephalitis in a US soldier returning from Saudi Arabia after the first Gulf war (Ferranti and Dolan 1993). Four other US soldiers also contracted the infection in Saudi Arabia following exposure to camels, sheep, and goats (Byrne 1997).

Another possible source of infection could be from wild ruminants, especially gazelles and oryx, which are held in captivity in wildlife research centers and as private collections in several farms around Riyadh and other major Saudi cities. To date, no report of coxiellosis in Saudi Arabian gazelles was available, whereas, only one previous record was made of

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*C. burnetii* antibodies in a herd of captive oryx tested more than 18 years ago (Greth et al. 1992).

The aim of this study was to investigate the prevalence of *C. burnetii* antibodies in two species of Arabian gazelles, namely the reem or sand gazelle (*Gazella subgutturosa marica*) and the idmi or mountain gazelle (*Gazella gazella*), as well as the Arabian oryx (*Oryx leucoryx*).

## Materials and methods

**Animals** A total of 555 animals of both sexes, comprising 227 Arabian sand gazelles, 232 Arabian mountain gazelles, and 96 Arabian oryx were sampled at King Khalid Wildlife Research Center (KKWRC) in Riyadh, Saudi Arabia, between January and June 2009. The Founders of the sand and mountain gazelles were obtained from the wild in Saudi Arabia, bred at KKWRC, and re-introduced in Mahazat As Sayd (near the city of Taif) in 1991 and Uruq Bani Mua'arid in the Empty Quarter desert in 1995 (Mohammed et al. 2010). The Arabian oryx, which became extinct in the wild since the 1960s, was obtained in 1978 from Phoenix Zoo, Arizona, USA, bred at KKWRC, and re-introduced in Uruq Bani Mua'arid in 1995 (Ostrowski and Bedin 2001). Sand gazelles attain sexual maturity in less than a year of age and are seasonal breeders, whereas mountain gazelles become sexually mature at 2 years and are polyestrous. The Arabian oryx reaches sexual maturity at more than 2 years of age and breeds throughout the year. None of these animals harbored ticks at the time of sampling. However, few *Hyalomma dromedarii* ticks are collected from them from time to time. There was also no direct contact between these wild ruminants and domestic animals, although camels were occasionally seen grazing close to the enclosures where the wild species were kept, while birds and rodents, which can also be potential sources of infection, were often spotted in the enclosures' vicinity.

**Sampling** A blood sample (7 mL) was collected from the jugular vein of each animal into a plain vacutainer tube (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) using a specifically designed buma to restrain the animals. The samples were allowed to stand for 4 h at room temperature, and the sera were separated from clotted blood by centrifugation at 1,500×g for 10 min, dispensed into clean 1.5-mL plastic tubes and stored at -20°C until tested. Samples showing hemolysis were discarded and replaced.

**Serological test** Screening for specific *C. burnetii* antibodies was carried out using an indirect ELISA immunoassay specifically designed to detect *C. burnetii* antibodies in the serum and milk of domestic and non-domestic ruminants by IDEXX laboratories (Chekit-Q-Fever,

Bommeli Diagnostics, AG, Bern, Switzerland). The test utilizes a horseradish peroxidase-labeled monoclonal anti-ruminant IgG conjugate that reacts with a wide range of domestic and wild ruminant species. The sera were tested in duplicate following manufacturer's recommendations. Briefly, each serum sample was diluted 1:400, and 100 uL was added in microtiter plates pre-coated with phase I and phase II *C. burnetii* antigens and incubated for 60 min at 37°C, followed by washing to remove unbound material using manufacturer-provided washing solution. One hundred microliter of peroxidase-labeled anti-ruminant IgG conjugate was then added to each well and incubated for another 60 min at 37°C, followed by washing. One hundred-microliter substrate was added in each well and incubated for 15 min, followed by 100 uL stopping reagent. Positive and negative reference sera (provided by the manufacturer) were included in 100 uL quantities in each test plate as controls. The degree of color that developed in the samples was measured as optical density (OD) at 450 nm using a spectrophotometric microplate reader, and the OD of the sample was compared with those of the positive and negative reference sera. The following equation was used to express the OD of the test samples as a percentage of the positive control (which was considered to be 100%):

$$\%OD \text{ of the sample} = \frac{100(S - N)}{(P - N)}$$

where *S* is the OD value for the test sample, while *N* and *P* are the OD values of the positive and negative reference sera, respectively. A good visual cut-off was observed at ≥40% OD, and the test samples were considered positive if the % OD value was ≥40 and negative if it was <40. Samples giving %OD values between 30 and 40 were considered doubtful and were re-tested.

**Statistical analysis** The  $\chi^2$  test (STATGRAPHICS plus 5.1) was used to analyze the relation between serological prevalence of *C. burnetii* and the species, sex and age. The effect of these factors on prevalence was determined by analysis of variance using a general linear model in SAS. The *P* value was set at ≤0.05.

## Results

Out of the total number of 555 animals tested, 99 (17.84%) were positive for *C. burnetii* antibodies. The serological prevalence varied significantly between the three animal species ( $\chi^2=73.08$ ,  $P<0.05$ ) and was highest in the Arabian oryx (Table 1). In both sand and mountain gazelles, females had higher prevalence than males, while the reverse was true in the Arabian oryx. On the other hand, although the overall

**Table 1** Serological prevalence of *Coxiella burnetii* antibodies in gazelles and oryx sera

	Males		Females		Overall	
	Total	Positive	Total	Positive	Total	Positive
Sand gazelle	87	8 (9.20%) a	140	29 (20.71%)	227	37 (16.30%) b
Mountain gazelle	92	5 (5.43%)	140	12 (8.57%)	232	17 (7.33%)
Arabian oryx	58	32 (55.17) a	38	13 (34.21%)	96	45 (46.88%) b
Total	237	45 (18.99%) a	318	54 (16.98%)	555	99 (17.84%) b

Data in the same row bearing lower case letters are significantly different ( $P \leq 0.05$ )

age effect was statistically significant, the differences between different age groups within each species did not show a consistent pattern (Table 2). Thus, while the highest serological prevalence in sand and mountain gazelles was recorded in animals aged 5–9 years, the oryx showed highest prevalence in younger age. On the other hand, none of the tested sand gazelles and oryx were serologically positive after the age of >9 years, while only two out of 22 mountain gazelles were positive at that age. No clinical signs were observed in the animals at the time of sampling. However, health records indicated the occurrence of a few cases of abortion among them in previous years.

**Discussion**

In this study, the serological prevalence of *C. burnetii* antibodies was determined in three species of wild desert ruminants indigenous to the Arabian Peninsula: sand gazelle, mountain gazelle, and Arabian oryx using the CHECK-Q fever enzyme immunoassay. This test detects antibodies against both phase I and phase II antigens (Rousset et al. 2007) and is currently one of the most commonly used serological tests for herd screening of *C. burnetii* antibodies in sheep (Berri et al. 2000, 2001; Schelling et al. 2003; Çekani et al. 2008; Kennerman et al. 2008; Karaka et al. 2009; Banazis et al. 2009), goats (Arricau-Bouvery et al. 2005; Rousset et al. 2007, 2009; Çekani et al. 2008; Khalili and Sakhae 2009) and cattle (Schelling et al. 2003; Seyitoğlu et al. 2006; Çekani et al. 2008; Banazis et al. 2009; Khalili and Sakhae 2009; Agger et al. 2010). It has also been used to screen *C. burnetii* antibodies in various

species of wild ruminants, including the field deer, *Ozotocerus bezoarticus* (Hernández et al. 2007), Spanish mouflon (*Ovis aries musimon*) (López-Olvera et al. 2009) and Dama gazelle (*Dama dama*) (Lloyd et al. 2010), as well as pseudo-ruminants, namely camels (Schelling et al. 2003; Hussein et al. 2008) and kangaroos (Banazis et al. 2009). The test was shown to have 100% specificity and 92–95% sensitivity relative to the indirect immunofluorescence tests (Bommeli 1997; Schalch et al. 1998; Arricau-Bouvery et al. 2005; Rousset et al. 2007).

To our knowledge, the present study is the first record of *C. burnetii* antibodies in the Arabian sand and mountain gazelles, and the second record in the Arabian oryx. In the earlier study on the oryx (Greth et al. 1992), the serological prevalence of *C. burnetii* was much lower than presently reported. Apart from the time lapse between the present study and that of Greth et al. (1992), the low prevalence reported by the latter authors could be partly due to the fact that they used complement fixation test, which is known for its low sensitivity as a Q-fever diagnostic tool. *C. burnetii* antibodies were reported in many other species of wild ungulates in the US (Sidewell et al. 1964; Enright et al. 1969; McQuiston and Childs 2002), Canada (Marrie et al. 1993), Europe (Stalis et al. 1996; Schröder 1998; Simmert et al. 1998; Martinov et al. 1989; Ruiz-Fons et al. 2008; Clemente et al. 2008), Japan (Ejercito et al. 1993; Yasumoto et al. 1997), Uruguay (Hernández et al. 2007) and the United Arab Emirates (Lloyd et al. 2010). In most of these animals, the prevalence of *C. burnetii* antibodies was comparable to, or even higher than that recorded in the present study. In the Hokaido deer (*Cervus Nippon yesoensis*) in northern Japan, the serological prevalence

**Table 2** Species by age distribution of *C. burnetii* seropositivity in gazelles and oryx (positive/total tested)

Age (years)	% positive (number of animals)			$\chi^2$
	Sand gazelle <i>G. subgutturosa marica</i>	Mountain gazelle <i>G. gazella</i>	Arabian oryx <i>Oryx leucoryx</i>	
<1	13.41(11/82)	11.76 (4/34)	60.00 (9/15)	13.58*
>1–5	10.77 (7/65)	3.97 (5/126)	60.00 (18/30)	
>5–9	26.40 (19/73)	12.00 (6/50)	38.30 (18/47)	
>9	0.0 (0/7)	9.10 (2/22)	0.0 (0/4)	

\*  $P < 0.05$

of *C. burnetii* antibodies was nearly 70% (Yasumoto et al. 1997).

The differences in the prevalence of *C. burnetii* antibodies between the three species of wild animals in the present investigation agree with findings in other wild ruminants (Ejercito et al. 1993; Yasumoto et al. 1997) and might indicate differences in susceptibility to *C. burnetii* between these species. The exceptionally high prevalence of *C. burnetii* in the Arabian oryx is particularly intriguing. These greatly endangered animals descended from a very small number of founders and were thus shown to exhibit a very low level of genetic variation for class II major histocompatibility complex (MCH) genes, which are believed to be important for pathogen resistance in vertebrates (Hedrick et al. 2000a, b). Whether MCH susceptibility applies to *C. burnetii* infection in the oryx is, however, unknown. The lack of significant sex-related differences in the prevalence of *C. burnetii* in the Arabian oryx is similar to previous findings in cattle (Nakoune et al. 2004) and camels (Hussein et al. 2008). On the other hand, the absence of serologically positive animals among oryx and sand gazelles aged more than 9 years could be due to the small number of animals available for testing in that age group, namely four oryx and seven mountain gazelles, as compared to 22 mountain gazelles of the same age that were tested. Besides, some authors argued that *C. burnetii* might sometimes be localized in the placenta or uterus of animals without eliciting systemic antibody reaction (Berri et al. 2001). In addition, studies on sheep, goats, and cattle indicated that age-associated seroprevalence of *C. burnetii* differed between different species of ruminants (Ruiz-Fons et al. 2010).

The absence of clinical signs in the present animals is consistent with the observation of Schröder (1998) that *C. burnetii* infection in wild ungulates was usually latent, but might become clinically manifested under stressful conditions. In that case, complications such as abortion, necrotizing placentitis, fetal dysplasias, and other reproductive problems might occur (Stalis et al. 1996; Lloyd et al. 2010).

The detection of *C. burnetii* antibodies in the present animals suggests that they are likely to become infected with *C. burnetii* and thus serve as sources of infection for humans handling them and possibly also for domestic animals in the vicinity. The transmission of *C. burnetii* from wild ungulates to humans has been documented, especially in people handling these animals in zoological gardens and wildlife research stations, hunters, and those living near forested areas. During an outbreak of Q fever among cervids in the Nüremberg Zoo, 26 zoo staff contracted the infection (Gaukler and Kraus 1974), while in Britain, a family of seven developed Q fever after hunting a deer and feeding its liver to the family's pregnant dog (Laughlin et al. 1991). Also, during an outbreak of *C. burnetii* abortions in a fallow deer farm near Stuttgart, Germany, 12 out of 13

in-contact persons were infected with *C. burnetii*, and two of them developed clinical disease (Simmert et al. 1998). Another outbreak of Q fever involving 25 (21.4%) out of 117 workers was reported in an experimental wildlife breeding station in Maldonado, Uruguay, in which the field deer, *Ozotoceros bezoarticus*, was identified as the main source of infection (Hernández et al. 2007).

Human Q fever has been described as holoendemic in some parts of Saudi Arabia (Gelpi 1966). While camels and other domestic animals are likely to be the main source of the infection in that country, the possible role of wild ungulates in spreading *C. burnetii* infection should not be overlooked. Serological tests do not always distinguish between exposure and actual infection, and it is therefore imperative that further studies be undertaken to isolate the organism and determine its shedding pattern in secretions and excretions of these animals. The populations of indigenous gazelles and Arabian oryx are growing rapidly in Saudi Arabia as a result of intensive breeding and re-introduction into the wild, thus, increasing the risk of disease transmission. Implementation of strict hygienic measures in breeding centers and private farms where these animals are kept is important to reduce 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 soiled with birthing fluids. Workers should also be advised on measures to protect themselves while handling these animals, such as wearing face masks, gloves and overalls (Lloyd et al. 2010).

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