



Coxiella Burnetii in Horses of Algeria: Seroprevalence and Associated Risk Factors

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ABSTRACT

The Q fever is a worldwide zoonotic disease caused by *Coxiella burnetii* (an obligate intracellular bacterium). This pathogen affects humans, ruminants, equines, carnivores, rodents, and birds. A cross-sectional study was carried out from March 2017 to May 2018 to assess the seroprevalence and identify the risk factors of *C. burnetii* infection in horses (*Equus Caballus*) residing in three districts of Algerian, namely Tiaret, El-Bayadh, and Ghardaia. Serum samples collected from 182 horses were analyzed via enzyme-linked immunosorbent assay (ELISA). Association of seropositivity with potential risk factors related to animals (e.g., age, gender, breed, housing, and presence of ticks), breeding characteristics (e.g., geographical localization, contact with animals), and environmental characteristics (i.e., presence of water source) was analyzed by univariate and multivariate logistic regression. An overall seroprevalence of 9.9% (18/182) was obtained. The univariate analysis of risk factors for *C. burnetii* seroprevalence demonstrated higher seropositivity in horses that had contact with small ruminants ($p=0.004$) and dromedaries ($p=0.002$) as well as in those living near a water source ($p=0.036$) and in El-Bayadh district ($p=0.005$). The multivariate logistic regression analysis indicated that the risk of *C. burnetii* infection was significantly higher in horses that were in contact with small ruminants (RR: 15.6). Algeria is endemic for Q fever in horses and prophylactic measures must be taken to reduce/prevent its transmission to animals and humans.

Keywords: Algeria, *Coxiella burnetii*, ELISA, Horses, Q fever, Seroprevalence

INTRODUCTION

Coxiella burnetii is a Gram-negative obligate intracellular bacterium that causes a zoonotic disease called Q fever, which affects humans, mammals, and non-mammals animals. Recently, this bacterium was classified in the order Legionellales, family Coxiellaceae (Bielawska-Drózd et al., 2013). Affected animals excrete *C. burnetii* in their body secretions, which could directly or indirectly contaminate the environment, animals, and humans. Parturient fluids from infected animals can contaminate the environment (Tissot Dupont et al., 1992). Although one of the most important sources of this pathogen is the birth products (i.e., placenta, amniotic fluid) of infected animals, dairy products of animals (e.g., milk and cheese) have been reported to transmit *C. burnetii* (Maurin and Raoult, 1999). The disease is transmitted to humans and animals not only through inhalation of infected particles from goats, sheep, and cattle but also by ticks that have been strongly implicated as vectors (Duron et al., 2015). In humans, Q fever can be asymptomatic, but acute forms with pneumonia or hepatitis have been also reported (Raoult et al., 1989; Angelakis and Raoult, 2010; Eldin et al., 2017). Additionally, endocarditis, vascular, and osteoarticular infections are associated with chronic Q fever (Raoult et al., 1989; Angelakis and Raoult, 2010). Since domestic ruminants are mostly incriminated in the transmission of *C. burnetii* to humans, many epidemiological studies have been conducted throughout the world. Infected ruminants with *C. burnetii* are mainly asymptomatic and the only detectable clinical signs include abortions at the end of gestation, usually 15 days before term (Rodolakis, 2004), stillbirth, premature delivery, birth of weak offspring, agalactia, and infertility (Cetinkaya et al., 2000).

To date, the role of horses in the Q fever epidemiology has not been deeply investigated. In experimental conditions, horses infected with *C. burnetii* developed fever, depression, enteritis, and/or bronchopneumonia (Zotov et al., 1956). Moreover, the isolation of *C. burnetii* from the placenta of aborted horses using molecular tests indicates a real involvement of the pathogen in reproductive disorders in this species (Runge et al., 2012; Roest et al., 2013). The

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role of horses in the transmission of *C. burnetii* to humans has been investigated. Some studies reported that people in close contact with horses, such as veterinarians and horseback riders, have more chances to be infected (Karagiannis et al., 2009; Palmela et al., 2012).

In Algeria, the zoonotic risk of *C. burnetii* has not been extensively investigated. In a study conducted by Lacheheb and Raoult (2009), the implementation of the immunofluorescence antibody test (IFAT) indicated a seroprevalence of 15.5% in humans, in Setif (northern Algeria). Recently, Ghaoui et al. (2018) identified 4 placentas affected with *C. burnetii* via IS1111 qPCR and IS30 qPCR as well as 3 seropositive individuals using IFAT out of 745 febrile spontaneous abortions in women. The findings of several studies on animals using serological tests indicated a seroprevalence range of 10-75% in ruminants and dromedaries (Yahiaoui et al., 2013; Agag et al., 2016; Khaled et al., 2016; Benaissa et al., 2017; Djellata et al., 2019; Bellabidi et al., 2020; Menadi et al., 2020). To our knowledge, there is no report about Q fever infection in horses in Algeria. Therefore, the present study aimed to assess the seroprevalence of *C. burnetii* in horses in three Algerian districts (i.e., Tiaret, El-Bayadh, and Ghardaia) and to identify the potential risk factors associated with *C. burnetii* infection in horses.

MATERIALS AND METHODS

Ethical approval

Risk assessment was submitted to and approved by the ethics committee of the Algerian Ministry of the Interior, the Local Government and the Algerian Ministry of Agriculture and Rural Development. Blood sampling was performed by a qualified veterinarian according to the guidelines for the care and use of the animal.

Study area and sample collection

The study was carried out in the three following Algerian districts (called Wilayas), namely Tiaret (North West), El-Bayadh (Southwest), and Ghardaia (South) as indicated in Figure 1. The study covers an area of 185648 km² spreading out between 28°54'30.87" and 35°38'50.86" North - 0°30'10.88" West and 4°58'54.03" East. These regions were chosen for their high concentration in horse breeding used for traditional events and production. The samples in the current study consisted of 182 horses randomly chosen from March 2017 to May 2018. The blood samples (approximately 5 ml) were taken through the jugular vein using vacutainer tubes. Sera were obtained by centrifugation at 3000 rpm for 10 min and stored at -20 °C until serological analysis.

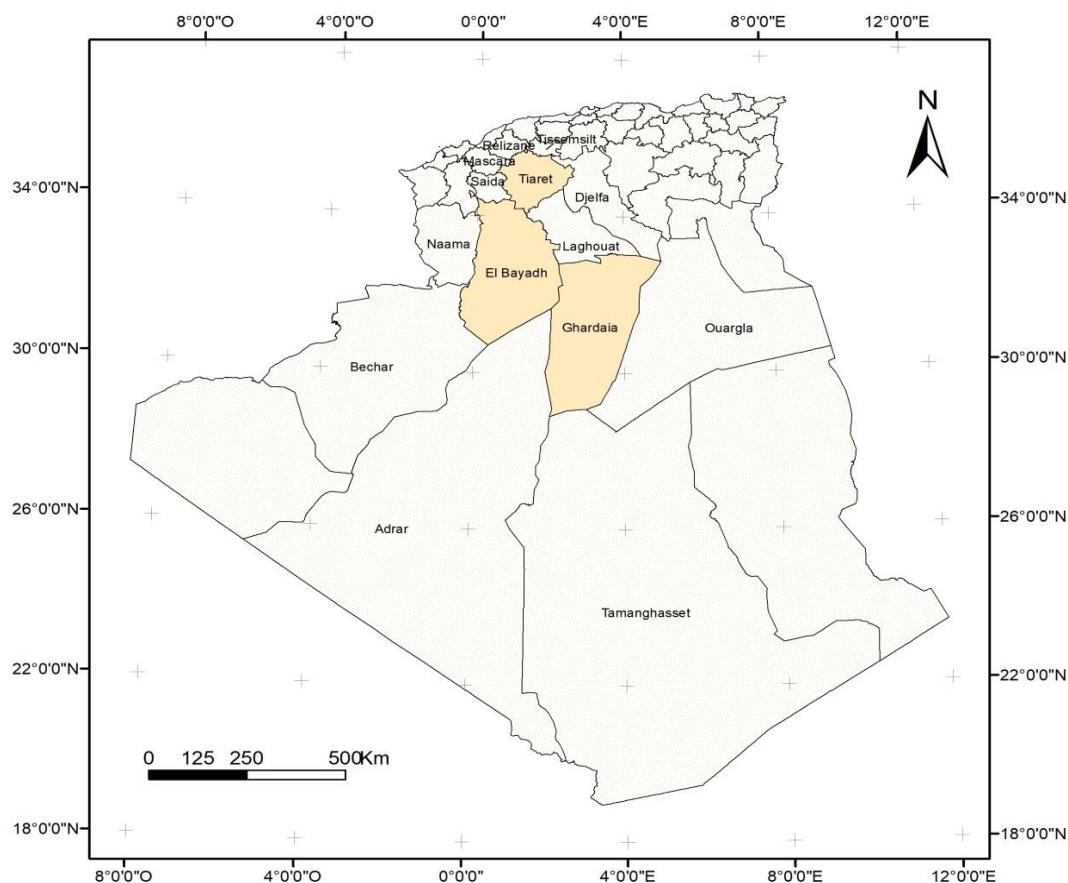


Figure 1. Location of Algerian districts for sample collection

Serologic test

The collected serum samples were tested for the presence of anti-*C. burnetii* antibodies via enzyme-linked immunosorbent assays (ELISA) using a commercial kit (ELISA-ID Screen® Q Fever Indirect Multi-species, IDVet, Montpellier, France) according to the manufacturer's instructions. The reaction was quantified by a spectrophotometer reading at 450 nm (Biotek Instruments Inc., USA). The results were reported in the optical density (OD) value. The interpretation of the results provided by the supplier (IDVet) is based on the value index. Samples were considered negative in case the OD was lesser than 40%, questionable when OD was between 40% and 50%, positive in case OD was higher than 50%. Two control serums (positive and negative) delivered by the manufacturer were used to validate the test.

Questionnaire

The recorded data was collected directly from the owners of the horses through questionnaire and included the demographic information of animals (age, gender, breed, housing, and presence of ticks), their breeding characteristics (geographical localization, contact with small ruminants and dromedaries), and environmental characteristics (presence of water source up to 1 km away).

Statistical analysis

The data were analyzed using R Studio (version 1.1.383, R Studio Inc., Boston, MA, USA). Seroprevalence was calculated by dividing the number of animals possessing anti-*C. burnetii* antibodies by the total number of the investigated animals. The explanatory variables were categorized as age (< 5, 5-10, > 10 years), gender (male, female), breed (Arabian, barb, Arab/barb, thoroughbred English), housing (box, stable), presence of ticks on animal body (yes, no), contact with ruminants (yes, no), contact with dromedaries (yes, no), presence of water source (yes, no), geographic location (Tiaret, El-Bayadh, Ghardaïa). Univariate analysis was used to analyze risk factors associated with seroprevalence. Multivariate logistic regression analysis was used to test the strength of association between the risk factors and *C. burnetii* infection using the generalized linear models (GLM). The best-fitting model to the dataset was constructed with a backward stepwise approach. The Akaike information criterion (AIC) was recruited to assess the multivariate model and the lowest AIC was used to select the best model. *P*-value less than 0.05 was considered statistically significant.

RESULTS

Seroprevalence

Out of 182 horses' sera examined, 18 (9.9%) samples were seropositive for *C. burnetii* antibodies.

Risk factors

Results of the univariate analysis of risk factors for *C. burnetii* seroprevalence in horses in the study area are summarized in Table 1. Proximity of horses with dromedaries and small ruminants was significantly associated with seropositivity ($p < 0.05$). Horses in contact with small ruminants and dromedaries had a higher risk of *C. burnetii* infection (15.4% and 17%, respectively), compared to those that had no contact with these two species. A higher risk of *C. burnetii* infection (14.1%) occurred in horses raised near watering points, compared to those that were farther. The close contact between watering points and horses significantly increased the seropositivity ($p = 0.036$). Seroprevalence was significantly different from one Wilaya to another ($p = 0.005$). This value was the highest in El-Bayadh (18.6%), followed by Ghardaïa (10.5%) and Tiaret (3.2%). Table 2 tabulates the obtained results of the logistic regression analysis. The potential risk factor related to the proximity with small ruminants indicated that *C. burnetii* infection was higher in horses that were in contact with small ruminants with a relative risk of 15.6% (95% CI = 1.87-130.02).

Table 2. The factors influencing the risk of *Coxiella burnetii* seropositivity in horses

Risk factors	Category	RR	95% confidence interval	p-value
Contact with small ruminants	Yes	15.6	1.87-130.02	0.006
	No	Ref	Ref	
Watering points	Yes	0.35	0.01 - 2.25	0.17
	No	Ref	Ref	

RR: Risk ratio

Table 2. The factors influencing the risk of *Coxiella burnetii* seropositivity in horses

Risk factors	Category	Total No.	Seropositive No.	Seroprevalence % (95% confidence interval)	P-value
Age	<5	59	7	11.9 (3.6 - 20.1)	0.66
	[5-10]	79	6	7.6 (1.8 - 13.4)	
	>10	44	5	11.4 (2 - 20.7)	
Gender	Female	173	18	10.4 (5.8 - 14.9)	0.308
	Male	9	0	0	
Breed	Arab	56	2	3.6 (0 - 8.4)	0.129
	Barb	72	8	11.1 (3.9 - 18.4)	
	Arabe-Barbe	54	8	14.8 (5.3 - 24.3)	
Housing	Box	76	5	6.6 (1 - 12.2)	0.205
	Stable	106	13	12.3 (6 - 18.5)	
Contact with dromedaries	Yes	88	15	17 (9.2 - 24.9)	0.002
	No	94	3	3.2 (0 - 6.7)	
Contact with small ruminants	Yes	104	16	15.4 (8.5 - 22.3)	0.004
	No	78	2	2.6 (0 - 6.1)	
Presence of watering points	Yes	99	14	14.1 (7.3 - 21)	0.036
	No	83	4	4.8 (2 - 9.4)	
Presence of ticks	Yes	106	13	12.3 (6 - 18.5)	0.205
	No	76	5	6.6 (1 - 12.2)	
Geographic localization (Algeria district)	Tiaret	93	3	3.2 (0 - 6.8)	0.005
	El-Bayadh	70	13	18.6 (9.5 - 27.7)	
	Ghardaïa	19	2	10.5 (0 - 24.3)	

No: Number

DISCUSSION

The *C. burnetii* has been reported in domestic animals, including sheep, goats, bovines, and dromedaries, (Dechicha et al., 2010; Yahiaoui et al., 2013; Agag et al., 2016; Khaled et al., 2016; Benaissa et al., 2017; Bellabidi et al., 2020; Menadi et al., 2020) as well as humans (Benslimani et al., 2005; Ghaoui et al., 2018) residing in Algeria. However, the present study was the first report of *C. burnetii* in horses and risk factors associated with *C. burnetii* infection in horses. Among 182 tested horses, a seroprevalence of 9.9% was obtained. This value is comparable to the pooled seroprevalence of 16% estimated throughout different areas of the world (Marenzoni et al., 2013). Higher rates of *C. burnetii* infection in horses were reported using molecular tests in different countries. Using PCR, a 42.2% rate of prevalence following abortions in horses was recorded in Croatia (Račić et al., 2014) and 7.7% was recorded in the aborted or non-aborted placenta in the Netherlands (Roest et al., 2013). Using loop-mediated isothermal amplification, a prevalence of 22.2% was observed in horse blood samples in China (Pan et al., 2013). Lower rates were observed via the use of complement fixation test in the horses' sera (0%) in Denmark (Agerholm et al., 2015) and by real-time PCR in aborted fetuses in France (1.5%) (Leon et al., 2012).

In Algeria, the observed seroprevalence in horses was lower, compared to the reported values in Cattle (10-29%, Dechicha et al., 2010; Agag et al., 2016; Khaled et al., 2016; Menadi et al., 2020), in dromedaries 71-75%, (Benaissa et al., 2017; Bellabidi et al., 2020) and in small ruminants 14-23%, (Yahiaoui et al., 2013; Khaled et al., 2016). The present study indicated that horses from El-Bayadh had a significantly higher risk of being infected (18.6%), compared to those of Ghardaïa (10.5%), and Tiaret (3.2 %). This discrepancy in the obtained results of various studies on the prevalence of *C. burnetii* can be due to many risk factors, such as the presence of tick vectors, climatic conditions, management practices, control measures, presence of infected animals in the horses' vicinity, and size of samples across different study areas. The prevalence differences were observed in small ruminants residing in different regions of other countries (Ullah et al., 2019; Aljafar et al., 2020).

Horses living near watering points were among the ones with the highest rate of infection. As reported, water proximity in Algeria allowed the proliferation of vectors, such as ticks, to transmit *C. burnetii* (Bessas et al., 2016; Leulmi et al., 2016; Aouadi et al., 2017). The contact of horses with small ruminants was a real risk factor for being seropositive to *C. burnetii*. The obtained result of the current study was indicative of the relative risk of 15.6% (95% CI = 1.87-130.02) for contracting the disease when horses were in contact with small ruminants. Small ruminants were considered as a reservoir and shedders of *C. burnetii* (Mertens et al., 2017). Since *C. burnetii* is resistant in the environment (Marrie and Raoult, 1997; Tissot Dupont et al., 1999), common spaces to horses and small ruminants become an important source of contamination. Our findings were in agreement with those of Menadi et al. (2020) and Keshavamurthy et al. (2020) investigating cattle, Maurin and Raoult (1999) and Rizzo et al. (2016) examining small ruminants. However, Benaissa et al. (2017) and Selim and Ali (2020) reported no significant relationship between Q fever infection in dromedaries and contact with small ruminants. In the current study, the contact of horses with dromedaries was identified as a risk factor for *C. burnetii* infection. Previous studies reported that dromedaries were important reservoirs of *C. burnetii* infection in the investigated areas (Benaissa et al., 2017; Bellabidi et al., 2020), and therefore, their proximity with horses increased the risk of contamination.

The findings of the current study indicated no significant association between the presence of ticks and Q fever infection which was supported by other researchers (Menadi et al., 2020; Selim and Ali, 2020). Previous studies, however, indicated that the presence of ticks increased the risk of infection by *C. burnetii* (Toledo et al., 2008; Benaissa et al., 2017). The role of ticks in the epidemiology of Q fever has been well documented, *C. burnetii* DNA was identified in about 40 species of ticks (Porter et al., 2011). It is well established that ticks play an important role in the sylvatic cycle of *C. burnetii* and in the contamination of humans and animals (Maurin and Raoult, 1999).

Age and gender in the current study were other insignificant risk factors, which were also reported in an earlier study on horses in South Korea (Seo et al., 2016). In ruminants, females were shown to be more infected with *C. burnetii* than males due to hormonal differences (Cetinkaya et al., 2000; Mazeri et al., 2013; Aljafar et al., 2020). Considering ruminants and dromedaries, aged animals are the most infected (McCaughy et al., 2010; Muskens et al., 2011), which could be due to the long duration of exposure to the pathogen. Also, there was no significant relationship between horse breed and Q fever infection. Although no exact explanation can be given, genetic predisposition is postulated as a reason. The type of horse sheltering was not associated with the infection of horses although keeping them in individual boxes could decrease the risk of tick-borne infections.

In this study, ELISA detected only IgG, thus it is difficult to differentiate between current and old infections, the use of more adequate tests in horses as molecular tests is highly recommended.

CONCLUSION

The obtained results of the current study indicated that *C. burnetii* was endemic in horses in the study areas. El-Bayadh with a seroprevalence of 18.6% was the most infected area. The contact with small ruminants and dromedaries, living

near water sources, and geographical location were identified as risk factors for *C. burnetii* seropositivity in horses. It is supported that horses can play an important role in the transmission of the pathogen to other animals and humans. Therefore, molecular characterization of *C. burnetii* strains is recommended to compare strains isolated from horses with those from humans.

DECLARATIONS

Author's contributions

Ansel Samir conceived the study design, carried out laboratory work, participated in data analysis and interpretation, drafted the manuscript. Benfodil Karima conceived the study design, participated in data analysis and interpretation, revised the manuscript. Miroud Kamel revised the manuscript. Mohamed-Cherif Abdellah carried out laboratory work, participated in data analysis and interpretation. Abdelli Amine participated in statistical analysis and interpretation and revised the manuscript. Kaidi Rachid carried out laboratory work and revised the manuscript. Ait-Oudhia Khatima conceived the study design, took part in the coordination and management as well as field studies, participated in data analysis and interpretation, and revised the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

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