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Concurrent seroprevalence of the zoonotic diseases Q fever and brucellosis and their association with abortion in small ruminants from Medea province, northern Algeria

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Abstract
Q fever and brucellosis are two important zoonotic diseases. They affect reproduction in small ruminants and have significant consequences for public health and the economy. This study aimed to investigate the presence of exposure to zoonotic pathogens, *Coxiella burnetii* and *Brucella* species, in small ruminants and to determine their seropositivity in the province of Medea, Northern Algeria. A total of 157 blood samples were collected from 17 flocks in Medea province from unvaccinated small ruminants. Only animals more than six months of age and that had stayed more than one year in the herd were sampled. Two indirect ELISAs were used to detect antibodies against *C. burnetii* and *Brucella spp.* This survey was conducted in sheep and goat flocks, indicating that antibodies were detected in 16.5% (95% CI, 10.8 - 22.4) for *C. burnetii* and 7.6% (95% CI, 3.5 - 11.8) for *Brucella spp.* This study provided seroprevalence data for two major zoonoses, Q fever and brucellosis, using the same small ruminant samples. Our results showed that *C. burnetii* infection was higher than that of *Brucella spp.* infection in small ruminants in this area. An intriguing result of the present study shows that co-infection was detected in the farm P in the Medea province. These findings are essential to implement a One Health approach to assess the incidence of these zoonoses in humans and to study transmission routes, particularly among people in direct contact with these animals.

Introduction

In many countries, particularly in Algeria, Q fever and brucellosis are zoonoses that are incorrectly diagnosed and underreported. The extensive contact between humans and animals increases the risk of zoonoses (1, 2). Q fever is caused by an obligate intracellular bacterium, *Coxiella burnetii*. Ticks are considered the main vectors for transmission of the disease, but the respiratory route, through inhalation of contaminated aerosols, is also an important route of transmission (3, 4). This pathogen survives environmental stressors, such as sunlight or desiccation, by developing spore-like forms (4). This infection could lead to certain clinical signs, such as stillbirth, late abortion, and the birth of weak offspring, but most of the time, there are no

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symptoms (5). The bacteria are excreted in vaginal secretions, fetuses, milk, and excrement after a reproductive problem (6).

Q fever is highly underestimated worldwide and often inadequately diagnosed due to poor quality and accuracy in countries such as Nepal and Bulgaria (7, 8). In the Netherlands, a study has shown that this disease could spread and become a serious public health problem (9).

Brucellosis is a zoonotic infectious disease caused by species of the genus *Brucella*, which poses a threat to public health; more than half a million cases are reported every year (10). The principal species of the genus *Brucella* responsible for abortions and infertility in farm animals is *Brucella melitensis* in sheep and goats (11). In humans, chronic infection may occur after contamination with *B. melitensis*. This bacterium is the most common cause of human brucellosis worldwide. The disease can also cause osteoarticular complications if not treated correctly (12). Moreover, abortions, infertility, and reduced milk and meat production result in considerable economic losses for the global animal industry (10). Brucellosis continues to be endemic in the Middle East, Africa, Central America, Latin America, and parts of Asia, while it is under control in Europe, Australia, and New Zealand (10). In sheep and goats, the bacterial species of the genus *Brucella*, specifically *B. melitensis* and *B. ovis*, are the most common causes of brucellosis in small ruminants, whereas *B. melitensis* is the most common cause of brucellosis in goats; in domestic sheep, B. ovis is responsible for a sexually transmitted infectious disease (13, 14).

In Algeria, variable seroprevalence rates for Q fever and brucellosis have been reported in small ruminants depending on geographical location. This demonstrates that brucellosis and Q fever are endemic in livestock (11, 15-18). Building on these observations, this study aimed to understand better how these infections spread in local herds, determine their seroprevalence, and detect any co-infections with these two agents, which are known to cause abortions and reproductive problems in small ruminants, while also representing a concern for public health.

Materials and Methods

Geographic and Climatic Factors

Medea province is situated in the heart of the Tellian Atlas, and its strategic position makes it a key transit area and a link between the North and the Sahara, on the one hand, and between the eastern and western high plateaus, on the other. The mountain range to the north rises to an altitude of over 1,000 m, while the plateau is at an altitude of over 600 m.

The climate of Medea province ranges from arid and steppe-like conditions in the southern areas to Mediterranean with cold, rainy winters in the mountainous north. The latter receives between 400 and 600 mm of rainfall per year, while the south receives less than 400 mm. Summers are hot and dry throughout the province (19). Ruminant farming is an important economic activity in the province of Medea, generally in the southern part, where the semi-arid climate is favorable for pastoralism. The principal species farmed are sheep, in particular the Ouled Djellal breed, known for its adaptability and resistance to steppe conditions (20).

Sample collection (sampling)

A total of 157 blood samples were collected from 17 flocks in Medea province from small ruminants unvaccinated against Q fever and brucellosis between September and December 2023. It is essential to note that the herd's composition has changed over time, and animals have not been individually marked. Only animals more than six months of age and that had stayed more than one year in the herd were sampled. Samples were taken from sedentary herds, some of which included goats, which graze in the vicinity of villages. These farms and animals were selected randomly.

Using a Vacutainer tube, a blood sample was collected from each animal, after which the tube was labeled with a code and accompanied by an

information sheet. A centrifuge was used to separate serum from the precipitated blood after centrifugation at 1,500 × g for 15 minutes. The serum was then divided into aliquots in clean 2 ml plastic tubes and stored at -18°C. Any tubes showing hemolysis were excluded from the study.

Serological Examination of Serum Samples

All serum samples were tested for antibodies against *Brucella* spp. and *C. burnetii* by indirect enzyme-linked immunosorbent assay (ELISA) using Innovative Diagnostics. Screen Brucellosis Serum Indirect Multi-species ELISA for the detection of antibodies against *B. melitensis*, *B. abortus,* or *B. suis* in small ruminants, cattle, porcine serum and plasma, and Innovative Diagnostics Screen Q Fever Indirect Multi-species ELISA for the detection of anti-*C. burnetii* antibodies in small ruminants, cattle, and humans (Innovative Diagnostics "IDVet", Grabels, France), according to the manufacturer's instructions. An ELISA reader a microplate was used to record the optical density values.

Animal rights

Animal welfare was a particular priority in this study. Sheep and goats were manipulated carefully and treated gently, without any brutality, in order to limit their stress. All blood samples were taken from the jugular vein by qualified personnel, with care and in accordance with good practice. No medications or painful procedures were employed. All procedures were very brief, lasting no more than a few seconds, and the animal was immediately released after sampling.

Table 1. Summary of serological results

Farm	No of tested animals	iELISA Positive Samples			
		*Brucella spp. (*except *B. ovis)*		*C. burnetii*	
		No of positive samples	Rate of positive samples %	No of positive samples	Rate of positive samples %
A*	12	0	-	4	33.3
B	10	1	10	3	30
C	6	0	-	0	-
D*	13	0	-	0	-
E*	4	0	-	1	25
F*	3	0	-	0	-
G	9	0	-	3	33.3
H*	6	0	-	1	16.6
I*	4	0	-	2	50
J*	13	0	-	3	23
K*	17	0	-	0	-
L*	13	0	-	0	-
M	7	1	14.2	0	-
N*	10	2	20	3	30
O*	10	2	20	2	20
P*	10	6	60	2	20
Q*	10	0	-	2	20
Total	157	12	7.6	26	16.5

**Presence of a goat in the flock

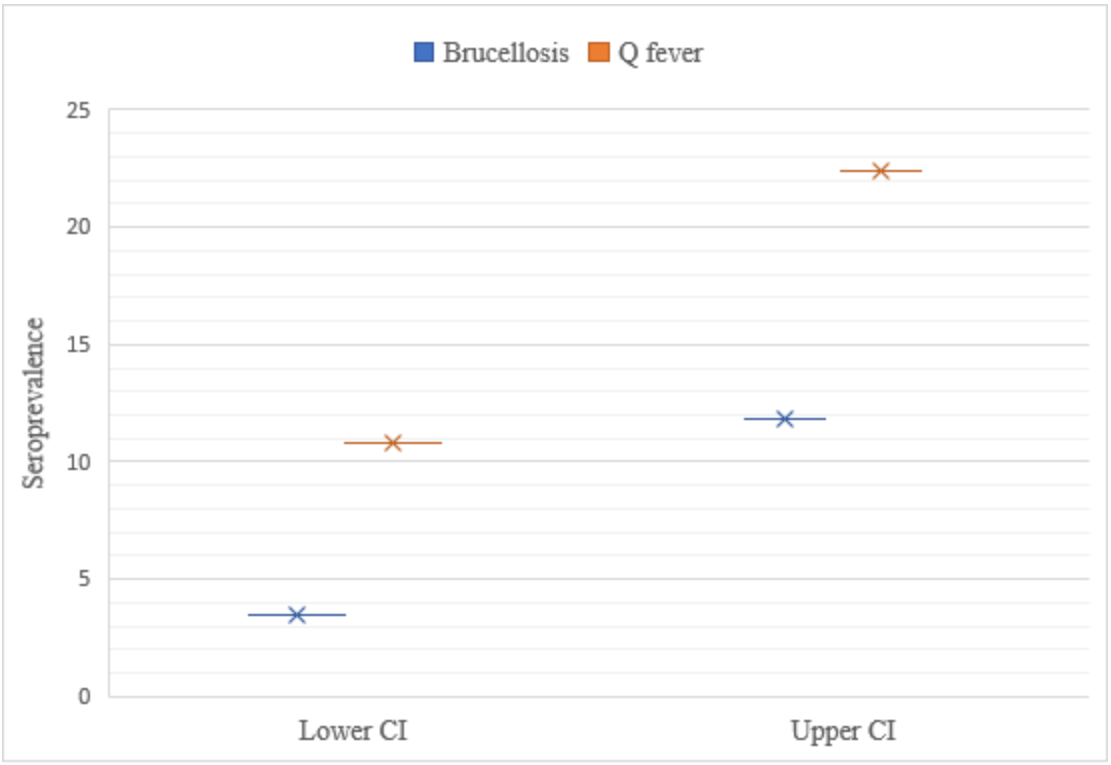

Fig. 1. Seroprevalence of Q fever and brucellosis in Small Ruminants (with 95% CI).

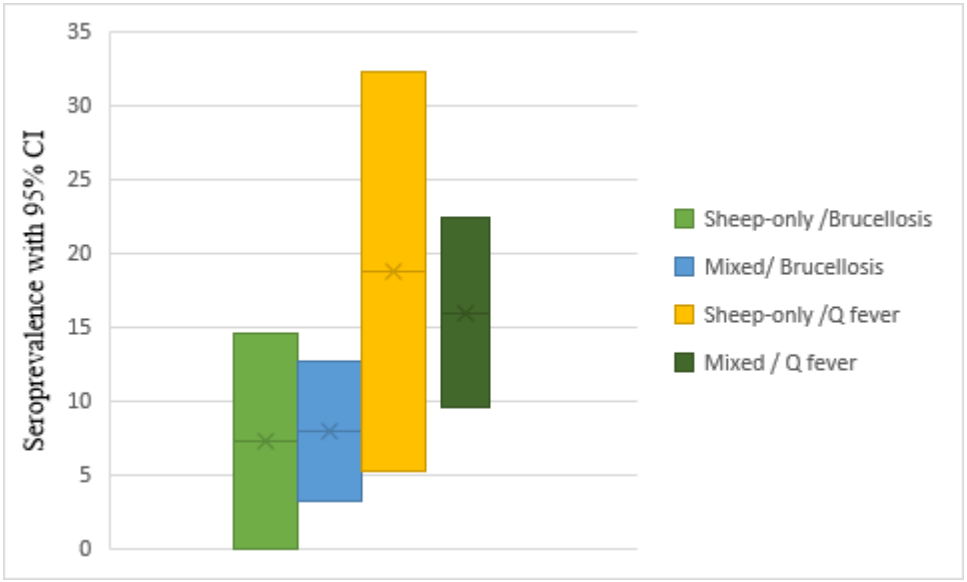

Fig. 2. Comparison of Q fever and brucellosis seroprevalence by flock type, with 95% CI.

Results

A total of 157 samples from 17 farms of Medea province were tested with commercially available ELISAs for the presence of antibodies against *Coxiella* and *Brucella*. The results of the serodiagnosis are summarized in Table 1. *Brucella* antibodies were detected in 12 animals (7.6%), and *Coxiella* antibodies were detected in 26 animals (16.5%). At the herd level, 5 farms (29.4%) were positive for brucellosis, and 11 farms (64.7%) were positive for Q fever; herds with one seropositive animal to ELISA were considered infected.

Seroprevalence rates differed significantly between the two infections: Q fever was 16.5% (95% Confidence Intervals CI 10.8—22.4), and brucellosis was 7.6% (95% CI 3.5—11.8) (Figure 1). The rate of Q fever seroprevalence in small ruminants was two to three times higher than brucellosis in this study.

Confidence interval plots provide a clear and rigorous way to compare seroprevalence and their confidence intervals across groups (such as sheep-only / mixed flocks, for both brucellosis and Q fever). This is highly suitable for emphasizing differences and statistical certainty. The prevalence of brucellosis is relatively low in both types of livestock farming, slightly higher in pure sheep herds than in mixed herds. In contrast, Q fever has a significantly higher seroprevalence, particularly in pure sheep flocks, exceeding 30%, compared to around 20% in mixed flocks. This kind of plot allows for a visually robust comparison between diseases and flock type (Figure 2).

Discussion

This study was the first in the province of Medea to provide seroprevalence data for two major zoonoses, Q fever and brucellosis, on the same small ruminant samples. Such an exhaustive comparison of the two zoonoses in small ruminants has never been done before in Algeria. In this study, the results showed a higher overall seroprevalence for infection caused by *C. burnetii* (16.5%) than infection caused by species of *Brucella* (7.6%) at the individual level, and 64.7% of farms were affected by Q fever, higher than the rate of 29.4% observed in farms for brucellosis. If a single animal in a herd was seropositive, the entire herd was considered to be affected by the disease. The two diseases are transmitted differently and survive in the environment in different ways, which probably explains the difference in their seroprevalence.

These observations about our results concerning Q fever, confirm those of a previous study conducted in the Medea region, which revealed a seroprevalence of 14.1% in small ruminants and the presence of at least one positive case in 58% of herds. (15). This is similar to the rate of our study; it showed that it is still circulating in the region and has been for years, despite specialist recommendations. There are several reasons for this persistence in the region: asymptomatic carriage by small ruminants, resistance of the pathogen in the environment, and inadequate preventive measures on farms. In addition, in the Ain Defla province (a neighboring province), the seroprevalence of Q fever among ewes was 24.9% and 66.7% at the animal and flock levels, respectively (17). Rates at the herd level are very similar, but at the individual level, seroprevalence remains very high. This can be explained by the choice of females, which remains an essential point. However, our findings indicate a higher seroprevalence compared to studies conducted in northeastern Algeria, which reported that four provinces revealed the presence of anti-*C. burnetii* antibodies in 35.06% of cattle herds and 8.73% of goats (21). This difference should be approached with caution, as their work is based on a larger sample. In addition, the selection of farms and animals, taking into account abortion history, may have influenced the data obtained.

It is difficult and delicate to compare the results of our study with previous serological studies in other

countries owing to assay type, criteria used for sampling, climate, and landforms of the region. However, higher rates of seroprevalence have been reported in Ethiopia. Seroprevalences were 25% and 28% in sheep, respectively (22, 23). Another study in South Africa showed an individual seroprevalence of 33.9% seropositivity with no significant risk factors (24). The ability of *C. burnetii* to spread rapidly over long distances is certainly due to its high resistance in the environment and the high level of concentration in the placenta and amniotic fluid during parturition (15). Persons in contact with animals represent a high risk for Q fever, a highly contagious zoonosis caused by *C. burnetii*. Transmission, which is often underestimated, is facilitated by the resistance of the infectious agent in the environment, potentially exposing urban communities (21, 25).

In Algeria, human Q fever is present, where it could cause a public health problem and should be considered as a differential diagnosis of non-specific febrile diseases (26). An interesting study shows that 3 of 70 patients (4.30%) were positive, including one PCR-positive (1.42%). All three patients had frequent contact with ruminants (27). As for the second zoonosis, the prevalence of brucellosis showed that anti-*Brucella* antibodies were detected in 7.6% of individuals, which is higher than the individual prevalence of 3.98% reported in bordering areas of southeast Algeria (18). Our seroprevalence was significantly lower than the brucellosis prevalence reported in both provinces of Medea and Sidi Bel-Abbès, northern Algeria, where 54.2% of serum samples were positive (11).

In Mali, 4.1% of animals were seropositive, which is comparable to the results observed in our study (28). This suggests that there are similar epidemiological dynamics, affected by conventional breeding practices, low immunization coverage, and limited vigilance systems. The circulation of *B. melitensis* strains in farms in the province of Medea has been confirmed, showing a high degree of genetic similarity to those involved in human infections in North Africa (11). These results confirm the zoonotic potential of this pathogen and its importance in public health, particularly for people in direct contact with animals, such as farmers, veterinarians, and slaughterhouse workers (11). Biovar 3 of *B. melitensis* predominates, showing genetic proximity to European strains, probably linked to economic exchanges. As in all Maghreb countries, brucellosis remains endemic, with a high incidence in humans, mainly associated with the consumption of unpasteurized dairy products and contact with infected animals (29).

An intriguing result of the present study indicates that both infections were detected at the same time on five farms B, N, O, P, and Q out of seventeen. One animal on farm P was found to be seropositive for both zoonoses at the same time, suggesting that it had been successively infected by the pathogens of both infections.

However, in flocks affected by both infections, competition between the two pathogens in sheep and goats could be occurring. In farms A, E, G, I, J, and Q in the province of Medea, where the prevalence of Q fever is higher (> 19%), the prevalence of brucellosis is null. In farm M, where the prevalence of brucellosis is higher (>14%), the prevalence of Q fever is null. The possible interference leading to mutual inhibitory effects should be further studied (30).

Sheep appear to be more susceptible to *C. burnetii* than goats, which could explain the higher rates observed in flocks consisting exclusively of sheep. In mixed herds, the cohabitation of species, farming practices, and hygiene levels influences the transmission of infectious agents: they can either promote the spread or, conversely, limit it, as is often the case with brucellosis. The latter spreads mainly through direct contact with abortions or genital secretions, while Q fever is transmitted much more easily through the air via contaminated dust. Finally, the persistence of old outbreaks or the

endemic circulation of *C. burnetii* in certain areas could also explain the differences observed between the two diseases and the types of livestock farming.

Conclusion

In conclusion, this study revealed the presence of antibodies against *Brucella spp.* and *C. burnetii*. Serological analysis indicated that the seroprevalence of Q fever was higher than that of brucellosis. Furthermore, co-infection was observed in one farm. To the best of our knowledge, the detection of antibodies against both *Brucella* and *Coxiella* in the same animal has never been studied in Algeria before. A notable result of the present study revealed that both infections were identified at the same time on five farms, out of seventeen. Future studies should include more investigations, such as those involving *Chlamydia abortus*, *Campylobacter fetus*, *Toxoplasma gondii*, and viral infections, like Border Disease Virus (BDV).

Given that brucellosis and Q fever have high seroprevalence rates in several herds, it is essential to integrate the "One Health" concept. This would help to assess the risks to humans and study how these diseases are transmitted, particularly among those who work with animals on a daily basis.

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Conflict of Interest

The authors declare that they have no competing interests.

Ethical Approval

The protocols was approved retrospectively by the Ethics Committee of the Institute of Veterinary Sciences, University of Blida 1, Algeria, under approval ID: UBI-IVS-2023-06.

Artificial Intelligence Statement

The authors confirm that this manuscript was produced completely by the research team, without the use of artificial intelligence systems.

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