Sero-epidemiological study of caprine brucellosis in Algeria

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Abstract

Our study aimed to investigate the prevalence of caprine brucellosis and identify factors influencing its persistence in Algeria. Over the span of a decade, from 2009 to 2018, a sero-epidemiological survey was undertaken, covering a significant population of 51,475 goats. The screening process involved the use of the card agglutination test to detect potential positive samples. Subsequently, positive samples were subjected to confirmation using the complement fixation test. The study revealed an average seropositivity prevalence of 14.7% over the ten-year duration. A significant difference (P<0.05) was observed between Southern and Northern provinces, registering respective rates of 20.2% and 6.9%. Furthermore, a pronounced effect of gender was evident (P<0.05), with females exhibiting a higher infection prevalence (15.6%) compared to males (7.1%). Similarly, a significant difference was observed concerning the age of animals, with aged individuals showing a prevalence of 15.8% in contrast to young animals at 8.5%. In our current investigation, we observed the persistence of caprine brucellosis across various provinces in Algeria. Consequently, there is a pressing need to establish and execute a comprehensive strategy aimed at combating and preventing this infection.

Key words: *Algeria; brucellosis; caprine; seroprevalence; risk factors*

Introduction

Brucellosis is an anthropozoonotic and ubiquitous disease (Acha and Szyfres, 2005). It is one of the major zoonotic pathogens and contagious diseases of ruminants in the world with considerable health and economic consequences (Bosilkovski, 2015). Besides the loss of animal productivity, brucellosis is a zoonosis of major health public importance; five out of the nine known *Brucella* species can infect humans and the most pathogenic and invasive species for human is *B. melitensis*, followed in descending order by *B. suis, B. abortus and B.* canis (Acha and Szyfres, 2003).

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Brucellosis in sheep and goats due to *Brucella melitensis* is the most important zoonosis that constitutes a serious hazard to public health. Successful campaigns have been carried out against small ruminant brucellosis based on screen-and-slaughter policy, and eradication has been achieved in many countries (Blasco and Molina-Flores, 2011). The disease has been eradicated in most Northern European countries, the United States and Japan. They are considered as unhurt of the infection because of the effective control strategies for veterinary prophylaxis established in these regions (OIE, 2018). On the other hand, in several African countries, this disease continues to be endemic, with a strong variability according to countries and regions in the same country (Aggad and Boukraa, 2006; Barkalla et al., 2014; Lucchese et al., 2016). Brucellosis is endemic in the Mediterranean basin, especially in the Northern African countries.

Algeria has experienced this pathology since the beginning of the 19th century until today and it continues to spread on farms, causing heavy economic losses (Benkirane, 2001). Until the 1980s, the epidemiological situation was not well known in Algeria and cases of human brucellosis were rarely reported or misdiagnosed despite an important animal reservoir. However, since the middle of 1980s, several outbreaks due to *Brucella. melitensis*, were reported in Ghardaia (Southern Algeria) Tlemcen (Western Algeria) and Setif (Eastern Algeria), resulting in more of human cases, this led the Public Health services to implement control and eradication measures, regarding animal as well as human brucellosis (Benhabyles et al., 1992).

In Algeria, goats are considered as the most important reservoir of human brucellosis (Benhabylles et al., 1992; Aggad and Boukraa, 2006; Lounes et al., 2014). In this country, brucellosis continues to spread in almost all regions of the country, with an aggravation of the situation, materialized by the increase in the number of human cases. It is a notifiable disease, and it is endemic and epidemic, mainly affecting (82% of cases) rural areas where domestic animal husbandry is prevalent, specifically Laghouat, Biskra, Tébessa, Tiaret, Djelfa, M'sila, and Khenchela. The number of human cases remains significant, around 7000 cases per year, leading Algeria to be ranked tenth globally in terms of annual incidence (Pappas et al., 2006). In this context, our current study aimed to investigate the prevalence of caprine brucellosis from a period ranging from 2009 to 2018 and to identify factors that may influence its persistence in Algeria.

Material and methods

Study area

The study was carried out in Algeria, located in North Africa, covers an area of 2,381,742 square kilometers, making it the largest country in Africa. It is bordered by Tunisia to the Northeast, Libya to the East, Niger to the Southeast, Mali, Mauritania, and Western Sahara to the Southwest, Morocco to the Northwest, and the Mediterranean Sea to the North. Algeria is divided into 58 provinces (wilayas) (Figure 1).

Climate

Algeria is a country in the subtropical zone of North Africa. Its climate varies greatly between regions (North-South, East-West). It is Mediterranean in type along the entire Northern coastline and the Tell Atlas (hot and dry summers, humid and cool winters), semi-arid on the high plateaus in the central part of the

Figure 1. Study area (Algeria location)

country, and desertic once the Sahara Atlas chain is crossed.

Data source

The epidemiological data were obtained from animal brucellosis cases reported between 2009 and 2018 in Algerian departments (WOAH, 2023).

Sample Collection

Blood samples were collected from goats by veterinary surgeons. The samples were collected from the jugular vein of the goats.

Transportation and Processing

The blood samples were transported to the corresponding Regional Veterinary Laboratories for each department. A total of 51475 goat blood samples were collected for analysis.

Serological analysis techniques

- Card Agglutination Test (CAT): This test is commonly used for the serodiagnosis of brucellosis. It detects the presence of antibodies against Brucella organisms in the blood serum. Positive reactions in the CAT indicate exposure to Brucella organisms.
- Confirmation of Positive Cases: Positive cases detected by the Card Agglutination Test were further confirmed using the Complement Fixation Test, following the protocol outlined by Alton et al. (1988). By conducting these serological analyses, the study aimed to determine the seroprevalence of brucellosis among goats in Algerian departments over the specified period. The data collected and analyzed would be valuable for understanding the epidemiology of brucellosis in the region and for informing strategies for disease control and prevention.

Statistical analyses

Three factors, (the year from 2009 to 2018, the sex, the age category, and the region) were included in our study. Likewise, three quantitative variables, number of clusters, number of positive and total tested animals, were considered as well. Before performing parametric tests

(Pearson test of correlation), the normality distribution was assessed using Chapiro-Wilks test. To explore correlation between the quantitative variables, the Spearman rank test was applied instead of Pearson correlation test because the data did not meet normality assumption. Then, the Spearman correlation coefficient "rho" was computed to indicate the strength of link.

The chi squared test of independence served to assess the association versus independence between categorical variables or factors. Data were analysed using the R statistical software (Version 4.3.1). All differences were considered as statistically significant when *P*-value<0.05.

Results and discussion

Prevalence of caprine brucellosis from 2009 to 2018

The data indicates that among 51,475 goats screened during the period from 2009 to 2018, 7,599 cases were seropositive for brucellosis found in 1,285 clusters.

The prevalence of caprine brucellosis fluctuated over the years, ranging from 8% to 24.9%. Despite these fluctuations, the average prevalence rate of caprine brucellosis during the study period was 14.7% (Table 1).

The difference was highly significant according to year (X-squared = 378.84, df = 9, *P*-value < 2.2e-16=2,2 x 10^{-16}). It should be noted that in Algeria, the national screening rate of animal brucellosis was considered to be minimal and insufficient for the detection of all positive cases (Agricultural and Rural Development Ministry, 2010). This minimal screening rate reported by the Ministry would be due to the breeders' unaware of the brucellosis dangers, or fear the positive animals to be slaughtered and therefore very poorly compensated. Thus, the breeders adhere to the screening program only to have the approval to sell milk. These rates of outbreaks and very high cases

Table 1. Prevalence of caprine brucellosis from 2009 to 2018

Year	Number of Clusters	Number of screened animals	Number of positive cases	Prevalence	*P*-value
2009	298	8920	1487	16.7	*P*<0.05
2010	241	10522	1811	17.2	
2011	66	2570	356	13.8	
2012	41	1553	148	9.5	
2013	44	1724	137	8	
2014	29	1242	171	13.7	
2015	49	2283	569	24.9	
2016	73	3358	440	13.1	
2017	164	7033	1128	16	
2018	280	12270	1352	11	
Total	**1285**	**51475**	**7599**	**14.7**	

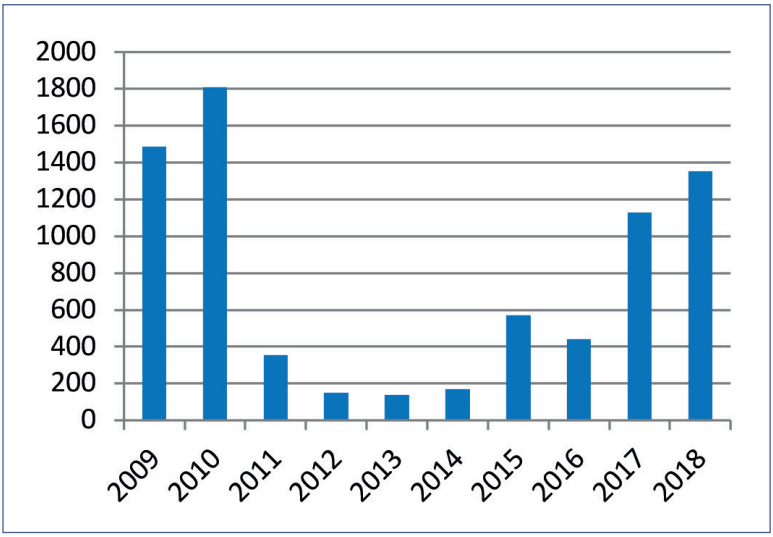

Figure 2. Evolution of National caprine brucellosis cases, from 2009 to 2018.

of brucellosis indicate that the Algerian livestock (all species combined) is very contaminated. Indeed, animal seroprevalence rates found in goats are significantly higher than those observed in sheep in several studies (Rechidi-Sidhoum et al., 2018). This is due to the fact that *Brucella melitensis* is a very pathogenic bacterial strain in the caprine species and can infect other animal species (Acha et Szyfres, 2005; Bosilkovski, 2015). Data reported by the Ministry of Agriculture and Rural Development (2009-2018) in Table 1, highlighted numbers of declining caprine brucellosis cases from 2011 from 10,522 in 2010 to 2,570 in 2011 to 2016 on goat farms (Figure 2). The caprine species is considered as the most important reservoir of brucellosis in Algeria and the main source of human disease (Lounes et al., 2014).

This variability in infection rates is not due to disease regression, but rather, to the numbers of herds detected annually. Indeed, despite the sanitary programs set up by the Government, the screening rate for animals remains very low, it is about 1%. This seems negligible given the large number of sheep and goats in the country. A significant difference (X^2= 1335.8, *P*-value < 0.0001) was obtained between brucellosis seroprevalence in south and north provinces with 20.2% and 6.9% respectively (Table 2).

Table 2. Caprine brucellosis prevalence from 2009 to 2018 according to the location

	North	South	Total
Screened	21125	30350	51475
Positif	1460	6139	7599
Prevalence	6.9	20.2	14.7
***P*-value**		< 0.0001	

This higher prevalence of caprine brucellosis observed in Southern regions of Algeria can be due to several factors: Climate and Environment (Southern regions of Algeria often have a warmer and drier climate, which may favor the persistence of the bacteria in the environment and increase the likelihood of transmission among animals), livestock Management Practices (traditional livestock management practices in Southern regions, such as transhumance, communal grazing, and extensive production systems, can lead to closer contact between infected and susceptible animals, increasing the risk of disease transmission), limited Veterinary Services (remote and rural areas, common in Southern regions, may have limited access to veterinary services, including diagnostic facilities and vaccination programs which can result in undetected cases of brucellosis and inadequate control measures, allowing the disease to persist within livestock populations) and finally, the socioeconomic factors, such as poverty and limited access to education and healthcare, may influence the prevalence of brucellosis where in some cases, farmers in Southern regions may lack awareness of brucellosis and its transmission routes.

Ramdani et al. (2022), indicated that estimated true herd prevalence was 27.95% (95% CI, 17.18-42.01) in the Southeast of Algeria. As reported by Khezzani et al. (2020), seroprevalence of caprine brucellosis in EL-Oued province (South Algeria) achieved 38.2%. The seroprevalence obtained in goats in Mostaganem (North Algeria) was 17.5% at the herd level (Rechidi-Sidhoum, 2018). Nehari et al. (2014) reported a prevalence of (3%) in El-Bayadh (Southern region of the country).

Comparing with some countries, it can be seen that in Tunisia, studies conducted in 1992 showed that the percentage of infected goats was 18% (Refai, 2002). Another study performed in 2009 reported a prevalence of 6.9% seropositivity among goats (HDIA et al., 2009). The serological survey done by Benkirane (2015) in Morocco revealed that 43% of herds were positive to brucellosis. The study of Douifi et al. (2021) showed that the Maghreb countries still record the highest brucellosis incidence rate in the world in both human and animal populations. The overall herd and individual seroprevalence of brucellosis in goats were estimated at 20.83%, 4.18%. The results obtained by Shakeel et al. (2020), mentioned that the goats were more ($P<0.05$) seropositive (19.5%) than sheep in five Districts of Punjab, Pakistan. In the study of Hajkazemi et al. (2020), it was shown that the seroprevalence of brucellosis at individual and herd-level were 4.0% and 33.2% respectively in Zanjan province (Iran). In the province of Bam (Burkina faso), Tialla (2022) noted that the individual seroprevalence was estimated at 4.3% and the "herd" prevalence was estimated at 40% in goats. Our results were

Table 3. Caprine brucellosis prevalence from 2009 to 2018 according to sex factor

	Male	Female	Total
Screened	5160	46315	51475
Positif	364	7235	7599
Prevalence	7.1	15.6	14.7
***P*-value**		< 0.0001	

significantly higher than those obtained by Fediaevsky et al. (2009), who found no antibodies against B. melitensis in serum collected from blood samples in sheep and goats in France. They pointed out that no infection with B. melitensis has been detected in France since the end of 2003. In addition, Rautureau et al. (2012) obtained the same results in France. In Jordan, Al-Majali (2005) noted that the prevalence of brucellosis seropositivity in goats was (27.7%).

Prevalence of caprine brucellosis according to sex

The seroprevalence was 15.6% and 7.1% in female and male, respectively during the year between 2009-2018. It was noted that higher number of females was screened than males (Table 3), and the seropositivity was also higher in females than males. A significant difference was observed between sexes of the animals in this study (X2 = 214.01, *P*-value < 0.0001). This trend can be attributed to the fact that the sample size of males was not as representative, thus reducing the likelihood of detecting many positive cases among them. Additionally, females were predominantly kept for breeding purposes, while males were typically slaughtered at 1-2 years of age. Consequently, females were at a significantly higher risk of contracting the infection due to greater exposure.

Our results were in agreement with several previous studies. The research conducted by Gompo et al. (2021) in Nepal revealed that the prevalence of brucellosis was higher in female compared to males. This could be attributed to the fact that female sheep and goats typically remain within the herd for longer periods since they are generally kept for breeding purposes rather than being slaughtered for meat. Females are often transferred between flocks for kidding, which may expose them to infected animals in new environments or introduce the infection to other flocks. Biologically, females are highly susceptible to *Brucella spp.* due to the presence of erythritol in their gravid uterus. Moreover, females were found to be more infected than males. Generally, there are very few males in the herds and in addition, the females abort which is one of the remarkable and visible signs of brucellosis (Tialla, 2022). Ali et al. (2015), reported same findings in Punjab (Pakistan) where low seroprevalence was recorded in males (3.03%) as compared to females (10.4%). Another study by Rivera et al. (2007) revealed seroprevalence for small ruminants (male 5%, female 9%) in Pakistan. However, Shakeel et al. (2020) noted that male animals were highly infected than females with a prevalence of 12.32% and 4.80% respectively. This result was supported by Saeed et al. (2019) who found a higher seroprevalence in male animals (7.4%) than in females (2.5%). Nonetheless, Tesfaye et al. (2021) and Ebid et al.

Table 4. Caprine brucellosis prevalence from 2009 to 2018 according to age

	Young	Adult	Total
Screened	7175	44300	51475
Positif	612	6987	7599
Prevalence	8.5	15.8	14.7
P-value		< 0.0001	

(2020) found no statistically significant difference in the frequencies of brucellosis infection between genders in their respective studies. This suggests that both male and female animals were equally susceptible to Brucella infection.

Prevalence of caprine brucellosis according to age

The study findings revealed a notable disparity in the prevalence of caprine brucellosis based on age. A significantly higher prevalence of 15.8% was observed in aged animals compared to young, which recorded a prevalence of 8.5% (Table 4). Statistical analysis indicated a significant difference among the various age categories ($X^2 = 200.39$, P-value < 0.0001).

Our data corroborate with those reported previously (Boukary et al., 2013; Akbarian et al., 2015) which showed that older animals were more susceptible to the infection than young and that could be due to low resistance against infection, greater exposure of older animals to the infection and hormonal dynamic in sexually mature animals. Koutinhouin et al. (2003) found that the prevalence of brucellosis was significantly associated with age. They had discussed that more the animal is getting older more it is likely to have been infected, to remain infected and to be dangerous to other animals. This increased the risk of infection with age logically corresponds to a greater probability of exposure to risk in older animals. In contrast, Shakeel et al. (2020), reported that no significant variation noticed between age groups (P>0.05). In the study of Gompo et al. (2021), it was shown that interestingly, age was not a significant risk factor for brucellosis in the goat population in the district. This may be because goat flocks were mainly maintained for meat production in Nepal, and most animals were sent to slaughter within a year.

Conclusions

To conclude, the findings indicate a high prevalence of caprine brucellosis in Algerian provinces, emphasizing the need to prioritize disease control efforts to safeguard both animal and public health, mitigate economic losses, and foster sustainable development in affected areas. Therefore, it is imperative to implement comprehensive measures aimed at preventing caprine brucellosis, such as enhancing veterinary services, raising awareness and education levels among farmers, implementing effective disease control strategies, and conducting research to identify region-specific risk factors and transmission dynamics. This elevated prevalence of caprine brucellosis presents significant challenges across animal health, public health, and socioeconomic fronts, necessitating improvements in diagnostic capabilities, promotion of farm biosecurity measures, and heightened public awareness regarding the disease and its transmission routes.

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Seroepidemiološka studija bruceloze koza u Alžiru

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Cilj je ove studije bio istražiti prevalenciju bruceloze koza i prepoznati čimbenike koji utječu na njezinu perzistenciju u Alžiru. Tijekom jednog desetljeća, od 2009. do 2018. godine, provedeno je seroepidemiološko istraživanje koje je pokrivalo značajnu populaciju od 51.475 koza. Postupak probira uključivao je uporabu testa s aglutinacijskom kartom za otkrivanje potencijalno pozitivnih uzoraka. Nakon toga, pozitivni uzorci su podvrgnuti potvrdi uporabom testa reakcije vezanja komplementa. Studija je otkrila prosječnu prevalenciju seropozitivnosti od 14,7 % tijekom deset godina. Značajna razlika (P<0,05) zamijećena je između južnih i sjevernih provincija, sa zabilježenim stopa-

ma od 20,2 %, odnosno 6,9 %. Nadalje, naglašeni učinak spola bio je očit (P<0,05), s tim da su ženke pokazale veću prevalenciju infekcije (15,6 %) u usporedbi s mužjacima (7,1 %). Zamijećena je I značajna razlika u svezi s dobi životinja, s prevalencijom od 15,8 % kod starijih životinja u usporedbi s 8,5 % kod mladih. U našem trenutnom istraživanju, zamijetili smo perzistenciju bruceloze koza u različitim provincijama Alžira. Posljedično, postoji hitna potreba za utvrđivanjem i provođenjem sveobuhvatne strategije s ciljem borbe protiv ove infekcije i njezine prevencije.

Ključne riječi: *Alžir, bruceloza, koza, seroprevalencija, čimbenici rizika*