RESEARCH PAPER

Serological diagnosis of brucellosis at the ruminants in Mostaganem (Algeria)

Nadra Rechidi-Sidhoum[1,2], Abdellatif Niar[3], Saïd Nemmiche*[1,4], Abdelkader Homrani[1,2]

[1]*Laboratory of Sciences and Techniques of Animal Production (LSTPA), Faculty of SNV, University of Mostaganem, Mostaganem, Algeria*

[2]*Department of Agronomy, Faculty of SNV, University of Mostaganem, Algeria*

[3]*Institut of Veterinary Sciences, University of Tiaret, Karman, Tiaret, Algeria*

[4]*Department of Biology, Faculty of SNV, University of Mostaganem, Mostaganem, Algeria*

Abstract

Epidemiologic investigations were conducted during one year to evaluate the seroprevalence of the brucellosis at the ruminants in the region of Mostaganem. In the cattle, 3106 sampling of blood provided from 479 dairy farms was examined by means of the Rose Bengale test (RBT) and complement fixation test (CFT).The seroprevalence of global herd and individual were obtained respectively 3.34% (95% Confidence Interval [CI]: 1.73-4.95) and 0.97% (95% CI: 0.62-1.31). The rates of infection were significantly elevated in males compared to females. However, there is no significant relationship between the rates of brucellosis infection (p=0.66) and the different zones studied. The second investigation using RBT was conducted in the rearing area of small ruminants and involved 450 sheep and 287 goats. A significantly different overall individual seroprevalence was observed between ovine races (2.22%; CI 95%: 0.86-3.58) and goats (5.23%; CI 95%: 2.65-7.80). On the other hand, at the herd level, no significant difference of overall seroprevalence was observed between these species. The order of the RBT rates are respectively 7.69% (95% CI: 1.21-14.2) for ovine races and 17.5% (95% CI: 5.72-29.3) for goats. These results showed clearly the high rates of infection at the ruminants.

* **Corresponding Author:** Saïd Nemmiche ✉ said.nemmiche@univ-mosta.dz

Introduction

The brucellosis is one of the major zoonotic pathogens and contagious diseases of ruminants in the world (Bosilkovski, 2015). Infectious disease of bacterial origin, it is strongly pathogenic in the breeding animals (Acha and Szyfres, 2005; OIE, 2008). It is a contagious, transmissible by various routes between animals of the same species and those of other species (Bosilkovski, 2015). It is characterized by an important reduction in the productions, which has a little encouraging effect on the economy, particularly that of developing countries. The disease, also transmissible from animals to the humans, is considered as a major zoonosis with considerable health and economic consequences (Mc Dermott *et al.,* 2013). Brucellosis 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, 2016). 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).

At present, no region of the country is saved by this affection which does not stop propagating in the various animal populations (bovine, ovine and goats) with sometimes epidemic fits in several regions (Benhabylles *et al.,* 1992; Aggad, 2003). Thus, in 2010, 8 445 human cases were notified (26 for 100 000 habitants) (INSP, 2011) and 6 779 cases in 2015 (OIE, 2016). Furthermore, the official statistical data of the Pasteur Institute of Algeria (IPA, 2015) report 193 cases confirmed bacteriologically on a total of 560 takings received in 2015. In any case, the brucellosis remains one of the priority concerns regarding health for numerous countries. There is good reason to remind that among tweezers, *Brucella melitensis* is the most pathogenic bacterium for humans (Acha and Szyfres, 2005) and that in Algeria, goats is considered as the most important reservoir of human brucellosis (Aggad and Boukraa, 2006; Benhabylles *et al.,* 1992; Lounes *et al.,* 2014; IPA, 2015).

Moreover, it is the cases of human disease that are revealing most of the time of latent foyers underestimated by animal brucellosis (Acha and Szyfres, 2005).

The official data relative to the animal brucellosis are still difficult to analyze, since the program of fight set up in 1995 by the Algerian authorities for the screening of animals concerns only farms with dairy bovine breeding (DSV, 2005). These are detected only occasionally. Generally, at the request of the breeder who wants to deliver the milk of its exploitation to the units of dairy transformation. And as a consequence in the appearance of case of human brucellosis, investigations are then conducted to find the origin of the disease in livestock farms. The epidemiological and sanitary monitoring follow-ups show that the average individual rates of seroprevalence on a national scale amounted to 2.24% during the first quarter of year 2015 (DSV, 2015). It is necessary to note that the small ruminants are not the object of systematic screening of the brucellosis and that the rates of infections to these species remain very high (DSV, 2015) and it is true in spite of the operations of vaccination organized in certain regions of the country (DSV, 2005, 2015). Furthermore, the investigations to determine the seroprevalences of the brucellosis in our country, are insufficient, concern only some wilayas, and are consequently not representative of the real situation (Aggad, 2003; Lounes and Bouyoucef, 2007). The objectives of this study were to investigate the seroprevalence of brucellosis in the dairy bovine exploitations and to have an overview of the degree of contamination of a cattle-breeding area of small ruminants.

Material and methods

Study area and animals

Mostaganem is a Wilaya (department) located in the northwest of Algeria and covers a surface of 2269 Km². Bordering four wilayas, it is endowed in the North of a maritime facade of 124.5 km. This region is characterized by a milde and temperate climate with averages of pluviometry ranging from 350mm on the plateau of Mostaganem to 400mm on the Mountains of Dahra.

The averages of temperatures between the cold and hot seasons vary from 6 to 31°C. The region presents a geomorphic structure consisting of trays, plains, valleys, and glacis distributed from east to west. The economy of the Wilaya is mainly based on the fishing, tourism and agriculture.

Among predominantly rural wilayas, Mostaganem occupies the second position after Adrar with respective rates of 61.89% and 63.51%, the national average being 34.06%. Due to its agricultural vocation and its dominant rural character, the breeding of the ruminants occupies an important part of the economy of this region.

Target population
The bovine cattle are concentrated in the low valleys of the West and the plateau of Mostaganem (DSA, 2016). The size of herd mostly ranges between 2 to 6 cows per farm and livestock breeding is generally semi-extensive. The animals are mostly grouped together during the day, in a common pasture, controlled by one shepherd. Cattle breeds in the region are generally pure breeds imported mainly from Europe and genetically selected for their high milk production. This type represents 59% of the herd. There are also so-called 'improved' cross-breeds (Mansour and Abbas, 2015; Si Tayeb *et al.,* 2015). These are the result of interbreeding between the races imported between them, or between the races imported before 1962 and very rustic local breeds with low milk production. Farms are often mixed. Vaccination against brucellosis is not common among the farmers. The ovine and goat populations occupy particularly the mountainous zones where grounds are damaged and the reduced routes. The values of the herd size are very heterogeneous (from 2 to 300 heads) and the breeding in the majority of the cases is of extensive type (DSA, 2016). We often observe exploitations with mixed breedings (two to three species together). The vaccination against the brucellosis is not practised in the region (DSA, 2016).

Study design and sampling
A cross-sectional study was conducted from June 2014 to May 2015 to assess the seroprevalence of brucellosis in ruminants in the wilaya of Mostaganem.

The first survey concerned 3106 blood samples from cattle over 2 years of age, from 479 dairy farms, selected according to systematic random sampling. The number of subjects required for the study was established for an expected prevalence of 3% (Lounes and Bouyoucef, 2007) and a relative precision of 20% (Toma *et al.*, 2010). The second investigation realized at the small ruminant was led in a cattle-breeding area of these species. Due to constraints related to a lack of human resources, especially the difficulty of gathering herds in a common place. The survey concerned only the farms whose owners had kindly agreed to have their animals be the object of this operation. The number of tested herds were 65 (450 individuals) for sheep and 40 for goats (287 individuals). The animals are over 6 months old.

Blood sample (10ml) was collected, in dry vacutainer tubes, from the jugular vein of small ruminants and caudal vein in cattle. The serum obtained after centrifugation were subjected to serological analysis techniques according to the recommendations of the manufacturing laboratories. The collected serum samples were stored at -20°C until screened for bovine brucellosis using Rose Bengal test (RBT). All RBT-positive sera were further tested by the complement fixation test (CFT).

Serological testing
Rose Bengal Test (RBT): The serum samples were screen using RBT antigen. It consists of a concentrated suspension of *Brucella abortus* (strain S99) dispersed in acid buffer (pH 3.65) and stained with Rose Bengal. It can detect antibodies to *Brucella abortus, Brucella melitensis* and *Brucella suis* in serum samples (Lillidale Diagnostics, United Kingdom). Briefly, a volume of 30 µl of pure serum to be examined and 30 µl of antigen, put at ambient temperature, are deposited side by side on an agglutination plate, then mixed rapidly and gently stirred. A serum is considered positive if any agglutination was observed after 4 minutes of agitation. Positive and negative controls were included in the test. Complement fixation test (CFT):

The antigen used is a concentrated suspension of *Brucella abortus* (strain S99) (IDEXX Montpellier,

France). It was used to all sera tested positive by Rose Bengal Test. A serum with a titre of 20 UIFC / ml (international units of complement fixation) or higher is considered positive.

Statistical analysis

The percentage of animal seroprevalence of the brucellosis was defined as the ratio of the number of infected animals to the total number of animals examined. The percentage of herd seroprevalence was defined as the ratio of the number of herds infected to the total number of herds tested (Toma *et al.,* 2010).

The relationship between the variables studied was measured using the Pearson Chi-square test and the Fisher test, for a significant threshold corresponding to α = 5%. The data analysis was realized by means of the software Microsoft Excel 2007.

Results and discussion

To express the overall seroprevalence results of brucellosis, the epidemiological units (individual) animal and herd (group) were considered. The study concerned a representative sample of 3106 bovine blood samples from 479 dairy farms in the Mostaganem wilaya. Overall seroprevalence rates are 0.97% (95% CI 0.62-1.31) at the animal level and 3.34% (95% CI 1.73-4.95) at the herd level (Table 1).

This represents high rates, especially at herd level. The study also reveals that the percentage of seropositivity is significantly different according to gender (p = 7.77 10^{-5}) (Table 2). Indeed, it is higher in males (16.0%, 95% CI: 1.63-30.4) than in females (0.84%, 95% CI: 0.52-1.17, OR: 22.3, 95% CI: 5.20-72.8). Finally, the results in Table 3 show that brucellosis is prevalent in the study areas.

The rates of 1.31% (95% CI: 0.34-2.27) are recorded for the Dahra Mountains, 0.93% (95% CI: 0.43-1.43) at the Plateau level and 0.85% (95% CI: 0.33-1.38) for the Valleys East and West, but there is no conclusive effect on the rate of infection per zone studied (χ2 = 0.82, p = 0.66).

Table 1. Distribution of seroprevalence of the brucellosis in dairy cattle.

Level	N	Number of Positive (Seroprevalence, %)	CI (95%)
Herd	479	16 (3.34)	1.73-4.95
Animal	3106	30 (0.97)	0.62-1.31

n=Number of animals tested, CI= Confidence Interval.

Table 2. Distribution of seroprevalence of the brucellosis in dairy cattle by sex.

Sex	n	Number of Positive (Seroprevalence, %)	CI (95%)	p (F)
Male	25	4 (16.0)	1.63-30.4	7.77 10^{-5}
Female	3081	26 (0.84)	0.52-1.17	

n= Number of animals tested, IC= Confidence Interval, p= probability, F= Fisher test.

Table 3. Distribution of seroprevalence of the brucellosis in dairy cattle by study area.

Area	N	Number of Positives (Seroprevalence, %)	CI (95%)	p (χ²)
Dahra mountains	536	7 (1.31)	0.34-2.27	0.66
Plateau	1398	13 (0.93)	0.43-1.43	
East and West Valleys	1172	10 (0.85)	0.33-1.38	

n= Number of animals tested, CI= Confidence interval , χ²= Pearson Chi-Square Test

The results observed in small ruminants are illustrated in Tables 4 and 5. Although there is no significant difference in overall seroprevalences at the level of herd (p = 0.20), the rates are particularly high in goats 17.5% (95% CI: 5.72-29.3) compared to sheep, where they are only 7.69% (95% CI: 1.21-14.2) (Table 4). According to the results obtained at animal level (Table 5), overall seroprevalence is significantly higher (χ2 = 4.83, p = 0.03) in goats (5.23%, 95% CI: 2.65-7.80) than in sheep (2.22%, 95% CI 0.86-3.58).

Table 4. Distribution of seroprevalence rates of the brucellosis in the herd.

Herds	n	Number of Positives (Seroprevalence, %)	CI (95%)	p(F)
Sheep	65	5 (7.69)	1.21-14.2	0.20
Goats	40	7 (17.5)	5.72-29.3	

n= Number of animals tested, CI= Confidence interval, F= Fisher test.

Table 5. Distribution of animal seroprevalence rates of sheep and goat brucellosis.

Herds	n	Number of positives (Prevalences, %)	IC (95%)	Valeur de p (χ²)
Sheep	450	10 (2.22)	0.86-3.58	0.03
Goats	287	15 (5.23)	2.65-7.80	

n= Number of animals tested, CI= Confidence interval , χ²= Pearson Chi-Square Test

The values found in dairy cattle at the scale of animals and herds are close to those observed in the Centre region of Algeria (0.81% and 3%), respectively at the animal and herd level (Lounes and Bouyoucef, 2007), and those found in a study carried out on the prevalence of the abortive infectious diseases of the cattle, which was of the order of 3.06% (Derdour *et al.*, 2017). However, high individual rates (10.4%) were obtained in the survey in the El Taref region (extreme northeast of Algeria) (Bouzid *et al.*, 2010). It is difficult to compare these results with those of the present study, because the context of the El-Taref survey was methodologically different. In fact, the animals were not randomly selected and the study's objective was based on the identification of certain reproductive pathologies in Black Holstein dairy cows. It's the same for seroprevalences observed in cows during a survey released in the wilaya of Tiaret and who are widely superior to those found in this study (Aggad and Boukraa, 2006). According to the sanitary bulletins of zoonotic emitted by the Ministry of Agriculture, the evolution of brucellosis in Algeria varies from one year to another and from one region to another (DSV, 2015). Indeed, according to the same source, the prevalence rates of the bovine brucellosis reached 2.24% at the animal level and 2% at the herd level in the first half of year 2015 in the seven wilayas of the zoning, which represents a high level of prevalence (Benkirane, 2001).

These data concern, in fact only a part of the country's regions and therefore are not representative of the reality on the ground. Furthermore, as noted above, the screening is not systematic for all herds and animals. Besides, these values must be revised upwards, because only the dairy cattle on farms approved by the veterinary services are controlled, thus it is very likely that the prevalence of brucellosis is even higher in some other farms. Values below those found in the present study are observed in Morocco. The national survey conducted from 2010 at 2011 showed an animal seroprevalence at the herd of 2.1% and 4.9% respectively. In this regard, it should be noted the absence of information on the serological tests and the sampling methods used (Ducrotoy *et al.*, 2015).

After year 2011, individual (33.5%) and herd (88%) rates, which are very high, were recorded in the north west of the country (Lucchese *et al.*, 2016).

These high values are most probably likely to the use of RBT as a single test, the modified protocol of which was not valid for use in cattle. In addition, the small-scale survey focused on dairy farms in which abortions were often reported. This difference can give some explanation by the low number of males recruited in the study. Indeed, they have a leading part in the reproduction and they are in much reduced number in the dairy exploitations. Frequently, they are absent for economic reasons. Nevertheless, they constitute an important epidemiological factor in the transmission of the disease by venereal way. It emerges from the present study that the effect zones in the wilaya of Mostaganem does not seem to have a significant effect on the bovine seroprevalence ($\chi2=0.82$; $p=0.66$). Seroprevalence is higher in the Dahra Mountains (1.31%, 95% CI: 0.34-2.27) compared to the Plateaus (0.93%, 95% CI: 0.43-1.43) and the Valleys (0.85%, 95% CI: 0.33-1.38). This is likely due to the fact that in this forest area, the density of goat herds is higher than in other areas, and the cohabitation of cattle with goats and sheep is common practice. Added to it, *B. melitensis* has been found associated with abortions in some Middle Eastern countries, where *B. abortus* is rare or absent (Benkirane, 2006). Thus, promiscuity of cattle with goats and sheep infected with *B. melitensis* should be considered as a significant risk factor related to contamination and the occurrence of the appearance of abortions in cattle in certain regions (Aggad and Boukraa, 2006; OIE, 2008). In their study, Aggad and Boukraa (2006) reported a variability of seroprevalences according to the studied zones. The rates recorded were 8% of the level of the Tiaret city, 11.8% in the north and 25% in the south of the region at the herd level. At the animal level, the percentages of infection were 1.85%, 0.95% and 3.99%, respectively, at the city of Tiaret, in the north and in the south. These values differ greatly between these geographical regions and are due according to the authors, the insufficiency of control of brucellosis in

Algeria generally, the small proportion of cattle controlled by the herd, in particular, and the movements of animals without any control measure. In Morocco, a study carried out on bovine brucellosis in the province of Sidi Kacem revealed that in the intensive irrigated zone, dominated by the presence of imported breeds, the seroprevalence rates (animal 0.3% and herd 2.7%) were higher than in the vast rainfall zone, where local and cross breeds predominate (animal seroprevalence 2.7% and herd 10.4%) (Yahyaoui, 2012).

The results are in agreement with those of the epidemiological survey conducted by Elandalousi *et al.* (2015) in northern Tunisia. Using an ELISA-indirect test, a seroprevalence rate of 3.37% was found in cattle herds. The authors report that this situation is the cause of abortions in these animals, despite the introduction of preventive health measures for decades, without limiting the disease. Especially since extensive and traditional type farms are in an area where sheep and goats are concentrated. These authors also indicate that 70% of cattle herds own small ruminants. In another survey conducted in Tunisia, a significantly elevated rate was observed with RBT in cows with clinical signs of abortion (31.3%) compared to the rate observed in females whose pregnancy and parturition were normal (7.8%) (Barkallah *et al.,* 2014). Moreover, although it is the same northern region of Tunisia and the purpose of the two studies conducted by Barkallah *et al.* (2014) and Elandalousi *et al.* (2015) concern the determination of the frequency of brucellosis in dairy cattle herds with abortive pathologies, the reported results are considerably different. Indeed, seroprevalence rates are low in the research carried out in Kalaat El Andalous of Ariana governorate and highly elevated in that carried out in the different regions of Sfax. In a study in Libya, 42% of infected cattle are reported in a concentrated population in a mountainous area in the north-west of the country. This string value attributes to the mild Mediterranean climate of the region investigated (Ahmed *et al.,* 2010). Infections with certain zoonotic infectious diseases such as brucellosis change over time and space, and infection rates vary from one region to

another or even from one year to another. This is confirmed, in real time, by the animal health information (OIE, 2016). Overall, the animal seroprevalence rates found in goats are significantly higher than those observed in sheep (Table 5) ($\chi 2$ = 4.83, p = 0.03 with an odds ratio of 0.41 (95% CI: 0). However, the difference obtained between the percentages of seroprevalences by species is not significant at the herd level (p = 0.20). This is due to the fact that *Brucella melitensis* is a bacterial strain highly pathogenic in the goat species and can infect other animal species (Acha and Szyfres, 2005; Bosilkovski, 2015). Furthermore, the context of farms in the Mostaganem zone is often mixed with a predominance of livestock, sheep in the plains and valleys, and goats in mountainous areas (DSA, 2016).

The seroprevalence obtained in goats is 5.23% (95% CI: 2.65-7.80) at the animal level and 17.5% (95% CI: 0.86-3.58) at the herd level. These percentages are at the animal level much lower than those found in goats in the central region of Algeria (13.4%) (Lounes and Bouyoucef, 2008), and close to those found in Tiaret (2.6%) (Aggad, 2003) and in El-Bayadh (southern region of the country) (3%) (Nehari *et al.,* 2014). At the herd level, the values are much larger than those found in goats in the El Bayadh region (10.14%), similar to those found in the central region of the country (31%) but considerably lower than those recorded in Tiaret (42%). In sheep, Aggad (2003) found infection rates of 1.42% and 43.5%, respectively, in animals and herds. High infection rates in the central region of the country may be due to herd size and the large number of animals sampled. In addition, the study covered 10 wilayas, some of which are known for their high density in sheep and goat farming. The latter species are also considered to be the most important reservoir of brucellosis in Algeria and the main source of human disease (Benhabylles *et al.,* 1992, Lounes *et al.,* 2014).

The high seroprevalences recorded in the region of Tiaret were explained by the high concentration of goats in this pastoral zone, the source of contamination of cattle and sheep farms by the tweezers.

It is also an area where transhumance is a common practice that takes place without any control procedure. The data reported by the DSV (2015) revealed the average infection rates in goat farms 14.45% in 2010, 6.6% in 2011 and 7% in 2015. This Variability in infection rates is not due to the regression of the disease, but rather, to the numbers of herds detected annually. Indeed, despite the sanitation programs in place, the rate of animal testing remains very low, it is around 1%. This seems negligible in view of the large number of sheep and goats in the country. Otherwise, it should be noted that in the sanitary bulletins issued by the services concerned, the number of households detected and those positive, are never reported. Similarly, no cases of abortion are reported (DSV, 2015). In Tunisia, seroprevalence studies carried out using the ELISA-indirect test in small ruminants in the northern region shows that the incidence of Brucella infection is lower in sheep (1.05%) and goat (13.2%) compared to our results. In Libya, Ahmed *et al.* (2010) makes the same observations as those of the present research concerning brucellosis seropositivity, which is higher in animal level, in goats (31%) than in sheep (24%). These strong values are to be linked to breeding methods. Indeed, the control of this disease in small ruminants would be effective in reducing infection in other livestock.

Conclusion

Our investigations on the seroprevalence of animal brucellosis have highlighted high infection rates in cattle. Furthermore, the results indicate a dispersal of brucellosis foci in this species in all areas of Mostaganem. Otherwise, this study confirms the presence of caprine and ovine brucellosis never reported previously in the region. It would be important that surveys be conducted on cattle to determine which bacterium *(B. abortus* or *B. melitensis)* are the most common and the source of infection in farms in the region. The control programs carried out in this direction by the health authorities are not very effective. In fact, the screenings are irregular and limited to livestock farms which, in order to be able to deliver their milk production to the processing units, are obliged to have a sanitary approval for their herds.

As a result, infection rates reported annually by health authorities are underestimated (DSV, 2015). Due to the high pathogenicity of the goat Brucella, both for animals and humans (Benhabylles *et al.,* 1992, Lounes *et al.,* 2014), the implementation of a rigorous and systematic screening of all farms and animals is more than necessary to reduce the incidence of this disease in herds and from there, its transmission to humans.

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