

SITUATION OF BRUCELLOSIS IN CATTLE FARMS IN A PASTORAL REGION OF ALGERIA (DJELFA) AND ITS IMPACT ON PUBLIC HEALTH: AN EXAMPLE OF DETECTION OF *BRUCELLA* ORGANISM IN FERMENTED RAW COW MILK « L'BEN »

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Abstract. Brucellosis is an infectious disease that spreads to humans and causes major economic loss. In addition, it poses a public health problem in Algeria and in many countries. In order to assess the situation of this disease in the region of Djelfa (Algeria) and to estimate its risk on public health, a study was extended from 2010 to 2018. In addition, it is accompanied by bacteriological analyzes on samples of "L'ben" fermented milk taken from certain outlets in the same region to find out the importance of brucellosis, as an infectious disease, as well as the risks to the health of consumers. The results showed that out of a total of 287640 cattle, only 4.42% were screened with a reported prevalence of seropositive cattle of 2.51%. In addition, the prevalence of bovine brucellosis varied from year to year. It was low in 2010 with 07 reported cases (0.67%), while it was high in 2016 with 57 reported cases (4.18%). In addition, for human brucellosis, the prevalence varies between 4.3% in 2013 and 20.25% in 2018, with an average of 11.11%. In parallel, the results of laboratory analysis have shown that the average prevalence rate of *brucella* spp in fermented milk "L'ben" was 77.78% and that the highest rate was observed in Messaad (100%) and Hassi Bahbah (100%). While the lowest rate was reported in Dar Chioukh (33.33%). These results reflect the risk posed to cattle by brucellosis, especially on public health, and the risk posed by the consumption of brucellosis-contaminated L'ben. This necessitates the implementation of a disease control and awareness program in order to avoid various accidents and significant economic losses. In addition, the early diagnosis of the disease helps preserve the health of the consumer thereafter.

Keywords: L'ben, *Brucella* spp., food hygiene, consumer.

INTRODUCTION

Brucellosis is mainly caused by *Brucella abortus* in cattle, which has a big effect on the economy, a reduction in milk production, abortions in the last trimester, a long calving interval (Ullah *et al.*, 2014). It is an ancient and one of the most widespread zoonotic diseases in the world, affecting both public health and animal production (Rahman, 2014-2015). In addition, it is one of the most common contagious and communicable zoonotic diseases with high rates of morbidity and sterility for life.

The intra / interspecies infection rate has increased considerably in recent years due to mismanagement and limited resources, especially in developing countries (Zahoor Khan and Zahoor, 2018).

Humans are infected through contact with infected animals or by eating food of animal origin. Therefore, brucellosis is a public health problem, particularly serious because human disease is often debilitating if it is not properly treated at an early stage. Brucellosis in animals is also a serious economic problem, causing considerable losses and restricting international trade in livestock. These reasons have led each country to put in place control measures, or at least to consider which action is compatible with their resources (Fensterbank, 1989).

Comparatively, the number of new human cases reported in Algeria followed a similar trend in animals, highlighting the impact of the animal reservoir on public health. The human incidence could reflect the true epidemiological situation of brucellosis in animals (Kardjadj, 2016).

The main objective of this study was to explore the prevalence rate of bovine brucellosis as a zoonotic disease in the province of Djelfa, the health risk that could be caused especially when consuming L'ben as a fermented dairy product preferred by the people of the region and finally the influence of the average annual temperature as a climatic factor involved in the incidence of this pathology. This work was directed towards future proposals for the prevention of this condition responsible for the significant losses on the one hand, and on the other hand, the necessary corrective measures to fight against this disease in order to preserve animal and public health.

MATERIAL AND METHODS



Fig. 1. Geographic location of the province of Djelfa (ANDI, 2013)

Study area

The province of Djelfa is located in the central part of northern Algeria beyond the southern foothills of the Tellian Atlas from the north, the chief town of Wilaya is 300 kilometers south of the capital. It is between 2 ° and 5 ° east longitude and between 33 ° and 35 ° north latitude. It is limited to the North by the Wilayas of Médéa and Tissemsilt, to the East by the Wilayas of M'Sila and Biskra; to the west by the Wilayas of Laghouat and Tiaret and to the south by the Wilayas of Ouargla, El Oued and Ghardaïa (Fig. 1) (ANDI, 2013).

Retrospective survey and study of the distribution of the disease

A total of 287640 cattle were examined for brucellosis in the various Daïras of the Djelfa region during the period from 2010 to 2018. In parallel, during the same period, a study was carried out on a total of 16002 human cases declared sick (human patients) to find out the risk of the disease on public health and the Daïras most affected by the disease in order to facilitate the selection of sampling sites for bacteriological analyzes. Data from cattle and human patients were collected, respectively, from the Department of Veterinary Services (DVS) and the Department of Health and Population (DHP) of the province of Djelfa, Algeria.

For human brucellosis, the data relating to the distribution of patients according to the Daïras were recorded.

Bacteriological analysis

The choice of farms

Three places (Messaad, Dar chioukh, Hassi bahbah) located in the province of Djelfa were chosen to carry out bacteriological analyzes in order to search for the *brucella* species in the L'ben, the dairy product most consumed in Algeria and specifically in the province of Djelfa. The choice was based on the number of populations and the number of positive cases for each location.

Sampling

In order to assess the contamination of the L'ben by *brucella* species, samples taken were collected from the outlets of the Daïras: Messaad, Dar Chioukh and Hassi Bahbah.

For information, the l'ben is a dairy product resulting from the transformation of raw milk by fermentation and it is consumed raw without any heat treatment.

The samples were put in sterile bottles and identified. The samples taken were then placed in a refrigerated enclosure and then transported under cold conditions to the regional veterinary laboratory in Laghouat, where the time between the collection and the first analyzes does not exceed 24 hours.

Detection of *brucella* spp.

The milk ring test was used according to the procedures by the regional laboratory of Laghouat. In the laboratory, the samples were taken out of the refrigerated enclosure and the reagents stored cold were left 30 minutes at room temperature in the laboratory before the start of the analysis.

After rigorous stirring of the sample, 1 ml was deposited by micropipette menu of a tip in a sight tube. Then, 30 µl of colored antigen for the ring test was added after moderate shaking. Finally, after mixing the contents of the tube on the vortex, the tube is brought for 45 min to 60 min in an oven at 37 ° C, then, the first reading was taken. Then, overnight (6 hours to 8 hours) at + 4 °C for confirmation.

For interpretation, if the cream ring is white, it means that the result is negative. On the other hand, if the ring of cream is colored, this confirms that the result is positive.

Statistical analysis

Prevalences and/or percentage were calculated by year, Dairas and sampling sites. The years of study, the Dairas, the sampling sites and the mean annual temperature were used as sources of variation.

The statistical tests were carried out using XLSTAT 2009 software. The chi-square test (comparison of several proportions) was used to compare the prevalence of screening and the prevalence of seropositive cattle according to the years of study. It is thus used to compare the prevalence of the number of positive cases with *brucella* spp. samples of the L'ben according to the sampling sites.

The correlation coefficient (r) and determination coefficient (R^2) were calculated from the prevalence of bovine brucellosis in each year to estimate the link between the disease and the annual average temperature.

Confidence intervals were calculated for the evolution of the prevalence of cattle screening per year and the prevalence of seropositive cattle (%) as well as the prevalence of human cases positive for brucellosis.

RESULTS AND DISCUSSION

Bovine brucellosis results

Bovine brucellosis screening status

Table 1 below represents the distribution of the number of cattle with the number of cattle screened and the screening rate for each year (2010 to 2018). The results obtained showed that the number of cattle varied between a minimum of 29100 head in 2010 and 35250 in 2015. However, the number of cattle screened was low in 2018 with 937 cases (0.33%; or: 937 cases out of a total of 287640 heads), whereas it was strong in 2014 with 3020 cases (1.05%; i.e.: 3020 cases out of a total of 287640 heads). In addition, the prevalence of animal screening varies between 2.97% in 2018 and 9.41% in 2014.

Statistical analyzes have shown the existence of a very highly significant ($p < 0.001$) difference between the prevalence of cattle screening according to the years of the study.

Prevalence of seropositive cattle

According to Table 2, between the period of 2010 and 2018, we noticed that the maximum number of animals screened was reported in 2014 with a workforce of 3020 cattle (0.77%; that is: 98 cases out of a total of 12716 heads). In addition, the maximum number of cattle which were declared seropositive for bovine brucellosis was observed in 2014 with 98 cases (0.77%; that is: 98 cases out of a total of 12716 head), while the minimum was declared in 2010 with 07 cases (0.06%; i.e.: 7 cases out of a total of 12716 heads). In addition, for the rate of animals affected, the percentages vary between 0.67% in 2010 and 4.18% in 2016.

In parallel, a very highly significant difference was recorded between the prevalence of seropositive cattle according to the years of the study ($p < 0.001$).

Table 1

Status of bovine brucellosis screening in the province of Djelfa

Years	Cattle numbers	Effective of cattle screened	Prevalence of screening per year (%)
2010	29100	1038	3.57
2011	29200	1234	4.23
2012	31350	1299	4.14
2013	30750	1154	3.75
2014	32080	3020	9.41
2015	35250	1532	4.35
2016	34400	1365	3.97
2017	34000	1137	3.34
2018	31510	937	2.97
Total	287640	12716	4.42
Average	31960 ± 2201.98	1412.89 ± 627.93	4.41 ± 1.93
CI (95%)	[31951.95 ; 31968.05]	[1401.98 ; 1423.80]	[4.34 ; 4.50]
SA	/	/	***

CI (95%): Confidence interval (95%); SA: Statistical analyzes; ***: $p < 0.001$

Table 2

Distribution of seropositive cattle in the province of Djelfa

Years	Number of cattle screened	Cattle seropositive	Prevalence of seropositive cattle (%)
2010	1038	7	0.67
2011	1234	14	1.13
2012	1299	23	1.77
2013	1154	26	2.25
2014	3020	98	3.25
2015	1532	42	2.74
2016	1365	57	4.18
2017	1137	-	-
2018	937	-	-
Total	12716	267	2.51
Average	1412.89 ± 627.93	38.14 ± 31.31	2.28 ± 1.22
CI (95%)	[1401.98 ; 1423.80]	[34.38 ; 41.90]	[2.21 ; 2.81]
SA	/	/	***

CI (95%): Confidence interval (95%); SA: Statistical analyzes; ***: $p < 0.001$

Relationship between the average number of bovine brucellic cases and the average annual temperature

Figure 2 reports the mean temperature values over the years of the study. According to the results, the temperature varied between a minimum of 14.7 ± 7.77 °C for the year 2018 and a maximum of 15.8 ± 7.33 °C for the year 2016. The average

temperature during the nine years of the study was 15.3 ± 0.39 ° C. Overall, the Djelfa region is characterized by a hot climate in summer and cold in winter.

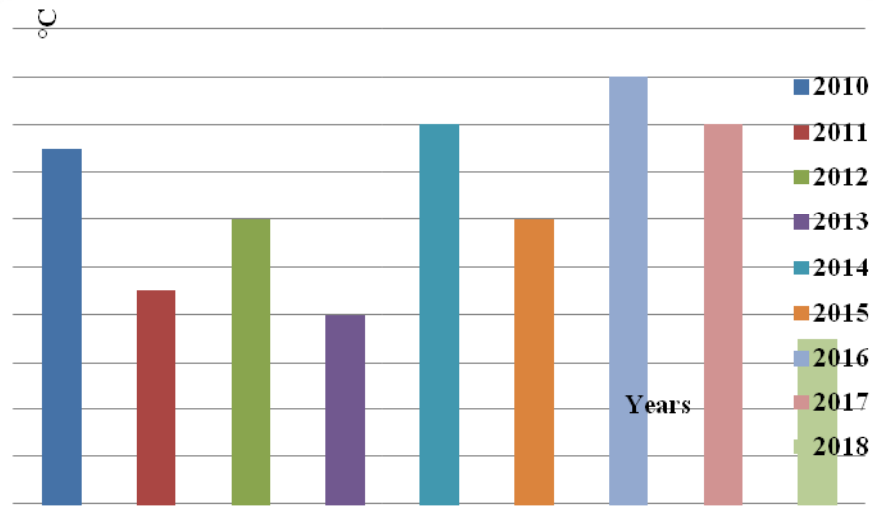


Fig. 2. Distribution of mean annual temperature values according to years of study in the province of Djelfa (2010 - 2018) (Infoclimat, 2018)

In parallel, Table 3 shows, for the cases of bovine brucellosis (BB) declared, the analysis between the disease and the average annual temperature. There is a moderate correlation for the disease (BB) compared to the average annual temperature ($r = 0.551$, $R^2 = 0.304$).

Table 3
Correlation between the average number of brucellus cattle cases and the average annual temperature

Years	Number of BB cases (seropositive)	TS	Relationship between settings	r	R ²
2010	7	15.5	BB-TS	0.551	0.304
2011	14	14.9			
2012	23	15.2			
2013	26	14.8			
2014	98	15.6			
2015	42	15.2			
2016	57	15.8			
2017	-	15.6			
2018	-	14.7			

BB: Bovine brucellosis; TS: Temperature; r: Correlation coefficient; R²: Coefficient of determination

Impact of brucellosis on public health

In order to assess the risk of consuming l'ben as a raw dairy product, we started with a survey to get an idea of the disease situation in humans and to discover the most endemic places (Daïras) by illness.

Survey results on human brucellosis

Overall results

Based on the recorded results of cases of human brucellosis in the province of Djelfa during the period from 2010 to 2018, we observed that this disease occupies an important place in this province, with a maximum recorded in 2018 with 3241 cases (20.25%) and a minimum in 2013 with 688 cases (4.30%). In addition, we noted in the province of Djelfa a low prevalence in 2012 and 2013 with 795 cases (4.97%) and 688 cases (4.30%), respectively, of human brucellosis compared to other years (Fig. 3).

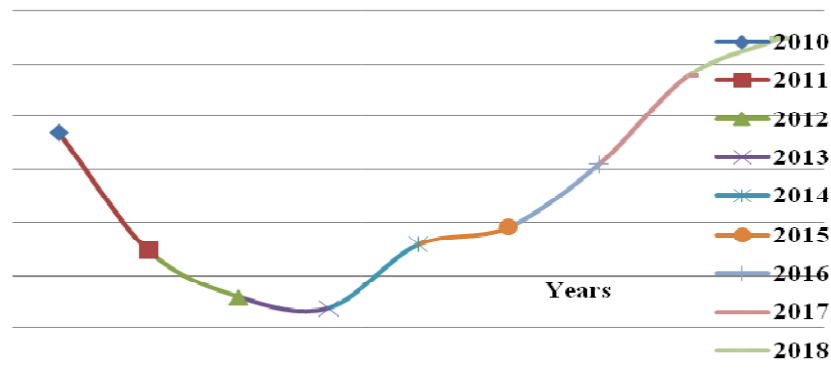


Fig. 3. Prevalence of human brucellosis in the province of Djelfa from 2010 to 2018

Prevalence of human brucellosis by Daïra during the year 2018

During 2018, it was noted that the number of brucellic inhabitants in the Daïras of the province of Djelfa most affected are in ascending order: Ain lbel with 244 cases (11.36%), Hassi Bahbah with 304 cases (14.16%), Dar Chioukh with 324 cases (15.09%), Djelfa 347 cases (16.16%) and Messaad 547 cases (25.48%) (Fig. 4).

Results of microbiological analysis

During the study period, the highest prevalence of brucellic L'ben was observed in Messaad (100%; i.e.: 10 positive cases out of a total of 10 samples) and Hassi Bahbah (100%; i.e.: 9 positive cases out of a total of 9 samples), which explains why all of the samples are contaminated with *Brucella* species. On the other hand, there are only two (02) samples (33.33%; that is: 2 cases out of a total of 6 samples) which are contaminated with *Brucella* species in Dar Chioukh (Table 4).

Statistical analyzes showed that there was a significant difference between the prevalence of the number of positive brucellosis cases and the sampling sites ($p < 0.05$).

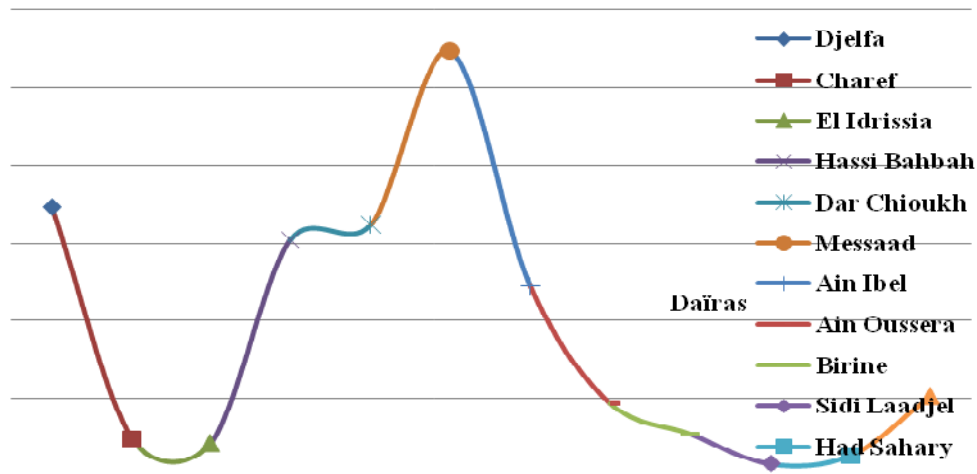


Fig. 4. Prevalence of human brucellosis by Daïra during the year 2018

Table 4

Prevalence of brucellosis in the L'ben according to the sampling sites

Sampling sites	Number of sampling	Number of positive cases	Prevalence of positive cases (%)
Messaad	10	10	100
Hassi Bahbah	9	9	100
Dar Chioukh	6	2	33.33
Total	25	21	84
Average	8.33 ± 2.08	7.00 ± 4.36	77.78 ± 38.49
CI (95%)	[7.51 ; 9.15]	[5.14 ; 8.86]	[68.32 ; 99.68]
SA	/	/	**

CI (95%): Confidence interval (95%); SA: Statistical analyzes; **: $p < 0.05$

The main objective of this study was to assess the prevalence rate of bovine brucellosis in the province of Djelfa in Algeria during the period from 2010 to 2018 and to investigate the implication of the average annual temperature as an extrinsic factor in the process of the evolution and transmission of the disease, on the one hand, and on the other hand, to know the impact of brucellosis on public health in order to propose corrective measures and guide future proposals for disease prevention.

The exploitation of the results obtained on animal brucellosis from the period from 2010 to 2018, the number of bovine animals screened and the number of positive animals declared and registered varies from year to year. In addition, it revealed a prevalence of cattle screening of 4.42% with an average of $4.41 \pm 1.93\%$. In parallel, the prevalence of seropositive cattle declared and recorded is 2.51% with an average of $2.28 \pm 1.22\%$.

At the national level, the present results concerning the prevalence of bovine brucellosis are similar to those reported by Kaaboub *et al.* (2019) in a study of

unvaccinated cattle, of which 280 (30 males and 250 females) belonged to 57 cattle farms with a prevalence rate of 2.5%. In parallel, they are significantly lower than those reported by Aggad and Boukraa (2006), in a study carried out in the province of Tiaret in western Algeria on 1032 cows spread over 95 herds to estimate the prevalence of brucellosis with an individual infection rate of 8.2% using the Bengal pink test. On the other hand, they are clearly lower than the results reported by Sidhoum (2019) in an epidemiological survey of animal and human brucellosis (Case of the Wilaya of Mostaganem) with a prevalence rate of 0.97% in cattle and those of Yahia et al. (2018), in Djelfa in Algeria with an average prevalence of bovine brucellosis over ten years of 1.4%.

Internationally, our results are significantly lower than those found by Ahmed *et al.* (2010) in a prospective study of *Brucella* seroprevalence which was carried out in north-western Libya (western mountain region) with a 42% prevalence rate of cattle were seropositive. On the other hand, they are clearly lower than those of Yahyaoui Azami *et al.* (2018) in a study on the prevalence of brucellosis and bovine tuberculosis in ruminants in the province of Sidi Kacem in northern Morocco from " a cross-sectional survey of 125 cattle with a brucellosis prevalence rate in cattle at an individual level of 1.9%. In addition, according to the Office International des Épizooties (2005), cited by Lounes and Bouyoucef (2009), in 2004, Tunisia declared only 2 cattle outbreaks.

In our study, the results obtained that the screening rate for cattle is low and insufficient in the province of Djelfa compared to the declared cattle population, in other words the screening for bovine brucellosis is only applicable on a limited number of cattle which makes it difficult to find all sick cattle and to fight brucellosis. This low detection rate is due to the non-cooperation of breeders who only subject their animals to screening by obligation of the authorities (to obtain authorization to sell milk); therefore, the majority of the animals screened are only dairy cows. The situation remains unknown for the rest of the beef industry. Farmers are not aware of the dangers of brucellosis, this is due to the lack of awareness; or for fear that positive animals will be slaughtered and therefore very poorly compensated (35% of the value of the animal). In addition, the veterinary services of the various inspections do not have means of transport to move to farms, especially in rural areas. Often it is the breeder to ensure the transport of veterinarians and sometimes to send samples to the laboratory and even to collect the results, which means that screening is done at the request of the breeder (Lounes and Bouyoucef, 2009).

According to, Sidhoum (2019), the influence of sex on the seroprevalence of the disease is significantly higher in males compared to females. In addition, according to the results of Kaaboub et al. (2019), older animals (≥ 8 years) were more infected. The seroprevalence of *Brucella* infection in cows with previous abortions was higher than in non-aborted cows. In addition, according to Yahia *et al.* (2018), bovine brucellosis persists in the province of Djelfa and it is necessary to set up a system to fight and prevent this infection.

The study of the relationship between the average number of bovine brucellosis cases available and the average annual temperature recorded for each study year revealed a moderate correlation ($r = 0.551$, $R^2 = 0.304$). The results obtained can be explained by several factors, in particular, the climate of the province of Djelfa

which is clearly semi-arid to arid with a continental nuance (ANDI, 2013). In addition, the amount of *Brucella* excreted in milk is quite low, but *Brucella* generally resist better than most other non-sporulating pathogenic bacteria to inactivation in the natural environment. Thus, when the conditions of pH (> 4), temperature and sunshine are favorable, these bacteria sometimes resist up to several months in water, in abortions and fetal envelopes, in cattle excrement, in the wool, hay and on equipment and clothing (Brisabois *et al.*, 1997).

Regarding the impact of brucellosis on public health, our survey results show a variation in the prevalence from 2010 to 2018. It is between a maximum of 20.25% in 2018 and a minimum of 4.30% in 2013 with an average of 11.11%. These results are clearly lower than those found by Ammam *et al.* (2018) in a study on the prevalence of human brucellosis in the southern region of Sidi-Bel-Abbès with a prevalence of 59.45% men against 40.54% of women and those of Tabet-derraz *et al.* (2017) in a study on the prevalence of human brucellosis in a farming region and who reported a prevalence rate of 33.4%. At the same time, internationally, the results remain significantly lower than those of Ahmed *et al.* (2010) in Libya, who found a high seropositive rate of 40%. Our results can be explained by the lack of awareness by the staff of the health and population management and the lack of consumer culture on the epidemiology and mode of transmission of the disease as well as the consumption of milk and raw dairy products.

According to Sidhoum (2019), in humans, the disease is endemic, more common in rural areas compared to urban areas. Very high rates are observed in people at risk (38%) and in blood donors (8%). Men are more affected by the disease than women. In addition, meat from slaughtered cattle tested positive for brucellosis can constitute a real risk of transmission to butchery staff and consumers, which requires that the meat of infected animals be analyzed before being marketed (Kaaboub *et al.*, 2019).

Brucellosis is a disease that remains endemic in some countries due to the escape of livestock from vaccination (Beji *et al.*, 2019). The distribution of the incidence of human brucellosis in the study region, according to the geographical origin of the patients made it possible to demonstrate that at the scale of the Daïras, the prevalence was higher in the Daïra of Messaad (25.48%) followed by the Daïras of Djelfa (16.16%), Dar Chioukh (15.09%), Hassi Bahbah (14.16%) and Ain lbel (11.36%). These results can be explained by the rural character of most of these places and the cultural human habit of consuming raw milk and dairy products like raw L'ben, which promotes the appearance of human brucellosis afterwards.

In addition, the transmission of *Brucella* infection to humans in Algeria depends largely on the animal reservoir and on several factors such as eating habits, methods of processing milk and milk products, social customs, conditions, socioeconomic status, animal husbandry practices and environmental health. Consumption of milk and raw milk products has been implicated in 85% of human infections (Kardjadj, 2016).

Following the previous results, the bacteriological analysis of the search for *Brucella* spp. in samples of the ben that were taken at points of sale located in the endemic and most popular Daïras of the province of Djelfa (Messaad, Dar Chioukh and Hassi Bahbah). The results obtained showed that the prevalence of *Brucella* spp. the

highest were observed in samples from Messaad (100%) and Hassi Bahbah (100%). This indicates the contamination of all samples with *Brucella* spp. On the other hand, the low prevalence was observed in Dar Chioukh (33.33%). Our results remain significantly higher than those of Aggad and Boukraa (2006) in the province of Tiaret in western Algeria, who found an individual rate of 4% of samples of contaminated milk.

Our results indicate that unpasteurized fermented milk could be a source of contamination. According to Sidhoum (2019), the mode of contamination by consumption of raw milk and its raw products derived from cattle or goats seems to be the most important, followed by direct contact with farm animals suffering from brucellosis. In parallel, according to Aggad and Boukraa (2006), the majority (89%) of humans infected with brucellosis had ingested raw milk or dairy products, mainly in spring and summer, which coincided with the birthing period and lactation of cattle and goats. In parallel, according to Touaref *et al.* (2014) in a study of 51 cases of brucellosis, hospitalized at the Guelma infectiology service in Algeria, the consumption of raw milk or its derivatives was the main risk factor for transmission (98%).

Furthermore, according to Garin-Bastuji and Verger (1994), cited by Brisabois *et al.* (1997), the survival of *Brucella* in milk and dairy products is linked to many factors, including the type of product, the water content, the temperature, the pH changes, the biological action of the other bacteria present, and the shelf life and conditions of the product.

In addition, the control and prevention of animal brucellosis requires respect for general hygiene in farms and the establishment at regional level of a control policy based on health and/or medical measures. All these measures cannot be truly effective without health education, training and the mobilization of the professionals concerned. Finally, no prophylaxis measure can be envisaged without a permanent identification of animals and herds and a strict control of their movements (trade, transhumance) (Brisabois *et al.*, 1997).

Finally, collaboration between the health department and the veterinary services department is important for the control of brucellosis in animals and thus eliminating transmission to humans. Training breeders in the effective implementation of sanitary and hygienic management practices for livestock after abortion contributes to reducing the spread of the disease in animals as well as in humans (Kardjadj, 2016).

CONCLUSIONS

The results of this study confirm that the prevalence of animal brucellosis varies from year to year. Infection with this disease is considered to be an important indicator of insufficient control of *Brucella* spp. which takes animals as its primary reservoir, which constitutes a risk to human health, especially in the case of domestic animals such as cattle, in the event of contact or consumption of raw products contaminated by this bacteria. In addition, the number of cattle screened varies from year to year and the screening rate is low and insufficient compared to the reported cattle herd in this province. This requires increasing the number of cattle screened for

each year in order to preserve the health of consumers, which is directly related to the eradication of bovine brucellosis through medical and health interventions.

At the same time, for human brucellosis, we found that the number of reported cases is higher in this province. In addition, research results for *Brucella* spp. in the L'ben as food more consumed by the Algerians, indicate the remarkable contamination of this foodstuff. This shows that the acidity of the L'ben studied does not destroy the *Brucella* spp. For this reason, awareness and popularization of breeders, consumers and other actors in the food chain is mandatory. In addition, it will be very useful to carry out systematic screening in all the farms in the country by general and specific laboratory examinations for confirmation. It is compulsory for cattle farms whose breeder has an agreement with the Department of the veterinary services (DVS) for the realization of the tests of early detection of brucellosis, which will contribute to better fight against the disease in cattle and in other animal species and preserving the health of consumers and thereby ultimately providing benefits for the entire economy of the country thereafter.

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