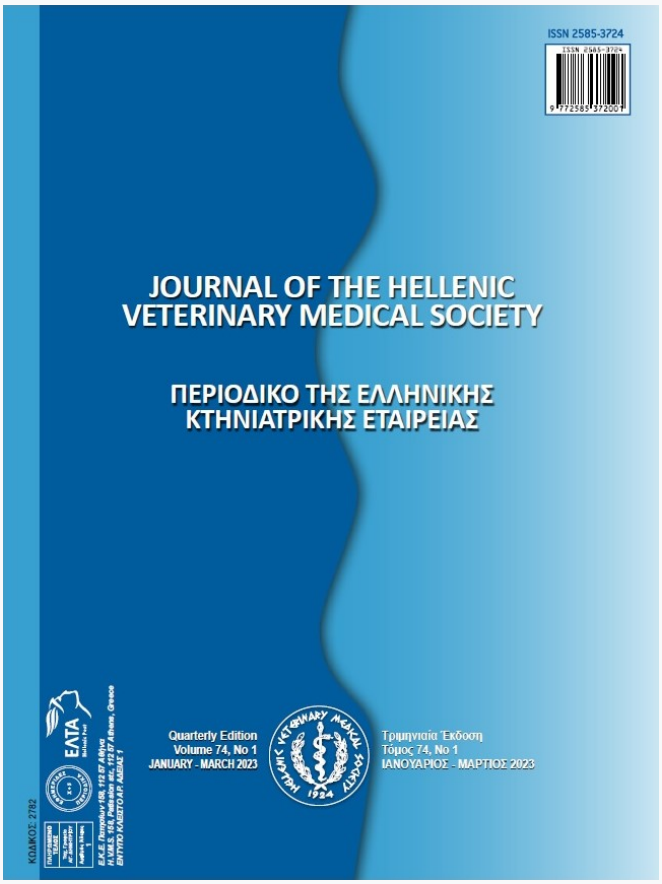
Journal of the Hellenic Veterinary Medical Society

Vol 74, No 1 (2023)



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doi: 10.12681/jhvms.29364

To cite this article:

Akkou, M., Titouche, Y., Bentayeb, L., & Ramdani-Bouguessa, N. (2023). Investigation of brucellosis in culled cows and professionals at El-Harrach's slaughterhouse in Algeria . *Journal of the Hellenic Veterinary Medical Society*, *74*(1), 5325–5334. https://doi.org/10.12681/jhvms.29364 (Original work published April 11, 2023)

J HELLENIC VET MED SOC 2023, 74 (1): 5325-5334
ΠΕΚΕ 2023, 74 (1): 5325-5334

Research article
Ερευνητικό άρθρο

Investigation of brucellosis in culled cows and professionals at El-Harrach's slaughterhouse in Algeria

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ABSTRACT: In Algeria, despite the control program (test/slaughter) applied since 1995, animal and human brucellosis incidence remains high over the last years. Our survey was carried out with,the aim of determining the insufficiencies of the applied prophylaxis plan, and assessing brucellosis hazards on professionals of slaughterhouse. On top of the 31 visits performed at the slaughterhouse of El-Harrach, blood sera and data associated with age, identification statute, race, type of slaughtering, pregnancy, and metritis presence were collected from 351 cull-cows. From the cooperative professionals of the same establishment, 31 sera in conjunction with the socio-epidemiological data were sampled. All sera were analyzed with Rose Bengal Test (RBT). The obtained results showed 0% prevalence in the slaughterhouse workers, and a global rate of 4.56 % RBT positivity in the cull-cows, within 5.55% prevalence in the pregnant ones. A rate of 50% (28.6 to 83.3%) daily frequency appearance of a sero-positive cow at the slaughterhouse of El-Harrach was established. In addition, 4.01% of slaughtered cows in the framework of healthiness slaughtering, were positive. While, no difference in prevalence related to race, neither to identification statute, nor for age, were recorded. Indeed, one can expect that the uncontrolled movement of animals can evoke a substantial factor limiting the effectiveness of brucellosis control programs.

Keywords: Brucellosis, cows, epidemiology, professionals, slaughterhouse.

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Date of initial submission: 28-01-2022
Date of acceptance: 19-10-2022

INTRODUCTION

Brucellosis isa re-emergent infectious zoonosis in many countries; it is characterized mainly, by a chronic debilitating state in human and, reproduction disorders in the animals (Lopez-Goni and Moriyon, 2005).The most common routes of human infection are related to occupational contact with animals, with transmission through inhalation of aerosols and contact with animal secretions. However, consumption of animal products can also lead to contraction of brucellosis (Cross et al., 2019).Thus, *Brucella* contamination of raw dairy products remains one of the main concerns for dairy product consumers in developing countries (Dadar et al., 2019).This disease has been substantially eliminated in several developed countries, including Australia, New Zealand, Japan, Canada, and some member countries of European Union, but it remains a major public health problem in the Mediterranean region, Middle East, Africa, Latin America, and part of Asia (Zhang et al., 2018).In the absence of systematic screening on occupationally exposed groups, 23.14 cases for 100 000 inhabitants were reported in 2007 in Algeria (INSP, 2007). Moreover, brucellosis prevails until now at an enzootic state in cattle, ovine, and caprine herds, even under multiannual campaign of control, based on screening and slaughter of the positive animals, and a vaccination program with *Rev 1* vaccine of sheep and caprine herds in 10 pilots' districts on the steppe province since 2006 (DSV, 2010).

The control campaign to bovines is applied only for the approved livestock (bovines carrying earrings). In 2009, 638 cases of brucellosis were diagnosed within 1333 positive bovines' heads; these latter were directed to slaughterhouses in the medical framework of elimination (DSV, 2010). Indeed, one should expect at least, a reduction in the incidence of brucellosis, in the breeding subjected to control program compared to those livestock escaping control.Professional exposure to brucellosis was reported in 49% of the patients suffering from brucellosis introduced to the Service Infectious Diseases of university hospital center of Oran, Algeria, for the period going from 2000 to 2007 (Boualem et al., 2009). At the state of our knowledge however,there is no published literature available on brucellosis status on slaughterhouse workers groups.

This study was conducted to assess at the slaughterhouse of El-Harrach (i) the sero-prevalence of antibodies to *Brucella sp* in cows presented to slaughter, (ii) the daily frequency appearance of a brucellosis infected cow within the abattoir, and finally (iii) the sero-prevalence of antibodies to *Brucella sp* in the slaughterhouse workers.

MATERIALS AND METHODS

Study design

The study protocol was approved by the local ethics committee (Ethics Committee of Mustapha Bacha Hospital). After obtaining written consent from the professionals of the slaughterhouse, a blood sample was taken from each cooperative person. The samples were analyzed for brucellosis and the obtained results were kept anonymous and strictly reserved for the present survey.

Study area

El-Harrach's slaughterhouse whose construction goes back to 1919, contains mostly a stalling surface of800m^2, a room slaughter of ruminants of 1200m^2 connected to a cold room and a slaughter room of equines. It is functional five days out of seven in the continuous day, morning (from 5h: 00 to 9h:00) and afternoon (from 12h:00 to 16h:00), Friday's morning and Saturday's afternoon. It provides 5% of beef and veal national production and, more than 12% consumed meat of the center area, making it the first national abattoir with regard to production in Algeria (DSV, 2010). The inspection of meats is guaranteed by three veterinary surgeons whereas the carcasses preparation is ensured by approximately sixty workmen.

Sampling design

The study was carried outin period of three months on cows presented to slaughter at El-Harrach's abattoir, Algeria. Thus, according to months, were picked the last two thirds of April, the first two thirds of May and the last third of June, in which visits were carried out.Because of the duration of the visit fixed to 5 hours; from 12h:00 to 17h: 00, in the absence of the bases survey, we were obliged to coincide the days of visit with the availability of the assistance then, to take blood from all found (alive present) cows. Hence, on top of 31visits, 351 cow's blood samples were taken. Moreover, from voluntary slaughterhouse workers of the same establishment, 31 blood samples in conjunction with their socio-epidemiological information's were also collected.

Epidemio-clinical data

For each cow, we collected information related

to identification status, age, race, state of pregnancy, metritis presence, and type of slaughter.We diagnosed metritis with open-cervix by examination of the fluid collected manually in the vaginal cavity, according to the scale of classification suggested by Williams et al. (2005). Moreover, only pregnancies of more than three months were taken in account with trans-rectal exploration then,a quantity usually of 4 mL of blood was taken either on the jugular veins or on the tail veins. In addition, certificates of elimination were inspected to determine whether cows directed to slaughter within brucellosis reason.

For the professionals, following an agreement with the principal inspector, the manager, a part of slaughterhouse' workers and the male nurse, an appointment for the realization of blood samples was fixed. It was under our supervision, that the male nurse took blood samples and that pre-tested questionnaire that took about 10-15 min was filled out by the assistant.

Laboratory screening of sera

The analysis of samples was carried out in the laboratory of biochemistry at the Veterinary High school of Algiers. After centrifugation of the blood samples for 5 minutes at 3000 round per minute, sera were transferred in Eppendorf and then frozen at -20°C until analysis.Sero-diagnosis of brucellosis was performed using Rose Bengal Test (RBT). The Rose Bengal test kits with *Brucella* antigen was kindly provided by BioSystems S.A. Costa Brava 30, Barcelona, Spain. The reagent was tested on a human serum, known positive to Brucellosis then, on another lacking in anti-*Brucella* antibodies.As described by OIE (2009) at the test procedure of Rose Bengal Test (RBT) we place 25-30 µL of serum sample and an equal volume of antigen. An applicator stick was used to mix the antigen and the serum. After that, the mixture was gently rocked for 4 min. The sample was classified positive if any agglutination was observed two times and negative, if no agglutination was observed.

Statistical analysis

The confidence intervals were calculated either by using formulas suggested by Toma et al.(2001), or the tables of the confidence intervals proposed by Jean (1996).According to the sensitivity of (0.95-0.99) and specificity of (0.91-1.00) shown by Domanech et al.(1980) in moderated zones, we calculated the individual true prevalence using the formula suggested by Toma et al.(2001).

$$TP = \frac{AP+Sp-1}{Se+Sp-1}$$

TP: True Prevalence, AP: Apparent Prevalence (calculated), Sp: Specificity, Se: Sensitivity

RESULTS

Animal sample characteristics

According to the registers data of slaughtering,1037 cows were reformed at the slaughterhouse of El-Harrach from April 1st to 30thof June. During our investigation period, into 31 performed visits, 351 blood samples were taken among 397 cows present in which the weekly evolution with regard the number of cows slaughtered is shown on the Figure1. Among the 81 [23.07%] cows carrying earrings, only 25 [7.12%] showed the Algerian mark type of identification, the Algerian identification were served to generate the map of source (Fig.2).

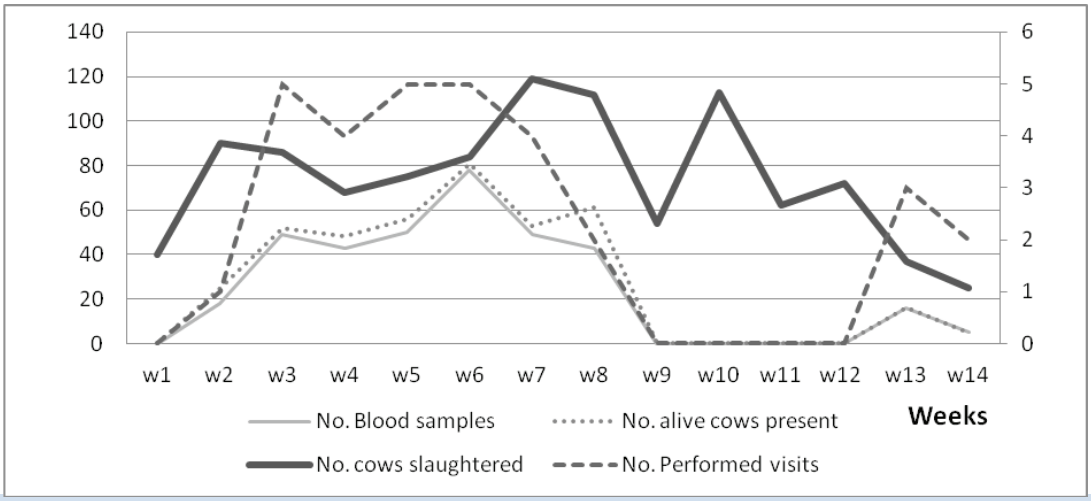

Figure 1: Evolution of slaughters, visits and sampling of the cull cows at the slaughterhouse of El-Harrach

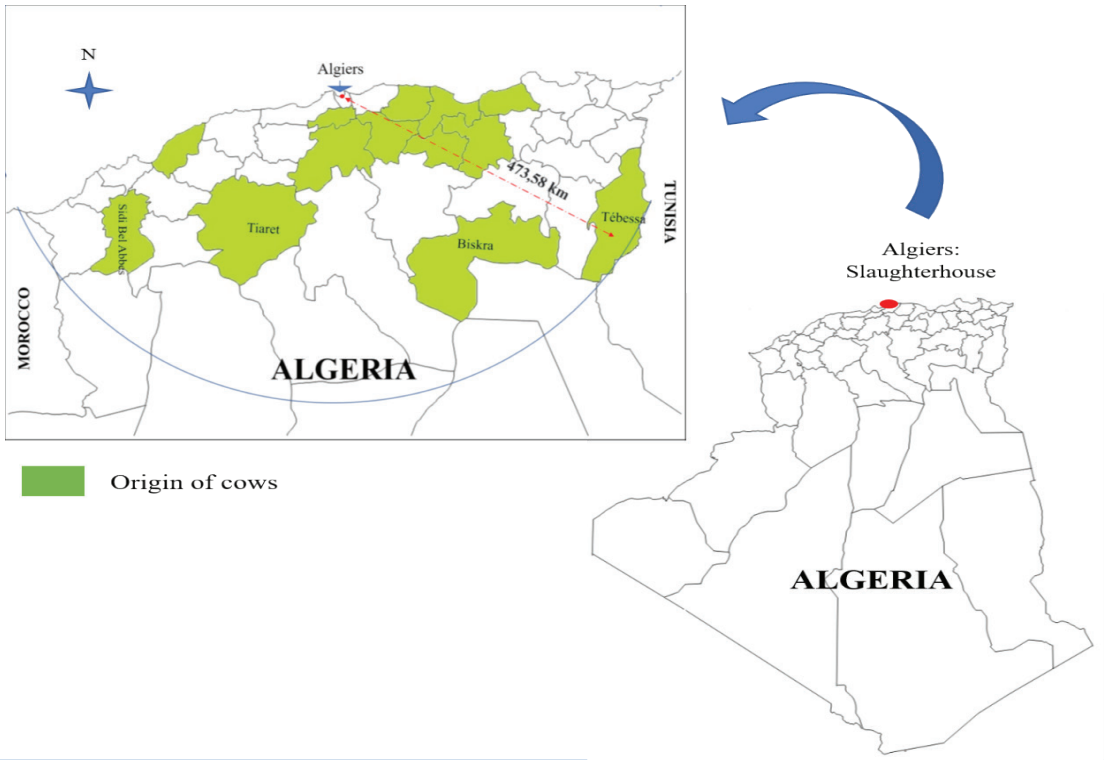

Figure 2: Origin of cows intended to the slaughterhouse of El-Harrach, according to earrings marks

Serology response to *Brucella sp.* in the cull cows

On 351 blood samples, the qualitative analysis with the RBT of sera resulting from the cull cows reveals 16 [4.56%, 95% confidence interval (CI) = 4.13- 4.99] positive cases with brucellosis. Moreover, a daily frequency appearance of an infected cow within the slaughterhouse of 50% (range 26 - 83): a 1 positive/2days/23±19 cull cow was recorded (Table 1 and 2, Fig.3).

Factors associated with seropositivity to *Brucella sp.*

After examination of the slaughtering certificates, we noticed that two cows were eliminated for brucellosis reasons. Thus, on 349 blood sera resulting from the allegedly salubrious reformed cows, 14 positive brucellosis cases, [4.01%, 95% confidence interval (CI) =3.99-4.03and true prevalence (TP) =4.30%] were diagnosed (Table 3). No significantly varied seroprevalence among identification groups, race and, pregnancy statute.

Table1: Seroprevalence of *Brucella sp.* in the cull cows and slaughterhouse workers

Epidemiological units	No. of samples	Positives to RBT	
		No.	%
Slaughterhouse worker	**31**	**0**	**0**
Total sampled cows	**351**	**16**	**4.55**
Cows sampled during April	131	4	3.05
Cows sampled during May	199	10	5.02
Cows sampled during June	21	2	9.52

Table 2: Monthly distribution of the daily frequency appearance of an infected cow at the slaughterhouse of El-Harrach

Months	Daily mean blood samples	Visits N	Positive cases	Appearance frequency of an infected cow	Frequency (%)
April	9.36±7.6	14	4	1case/ 3.5days/33±27 cows	28.57a
May	16.58±11.3	12	10	1 case / 1.2days/20±14 cows	83.33b
June	4.2±3.3	5	2	1 case/ 2.5days /10.5 ±8 cows	40a

There is a significant difference where we find two letters different

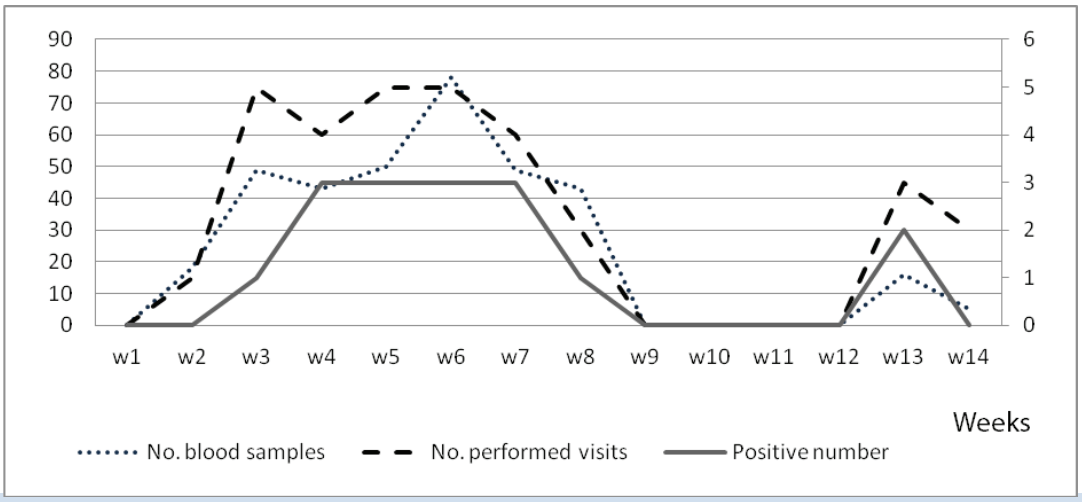

Figure 3: Evolution of brucellosis cases numbers, visits number and the sample size at the slaughterhouse of El-Harrach

Table 3: Variability factors of brucellosis prevalence at the salubrious category of the cull cows

Risk factor	Codification	No. of positive cases	No. Samples	Prevalence %	P
Identification	Identified	3	79	3.79	
	Unidentified	10	206	4.85	p>0.05
	Perforated ear	1	64	1.56	
	G1-IMP	1	36	2.77	
Race	Local	2	53	3.77	p>0.05
	Crossed	9	174	5.17	
	Not classified	2	86	2.32	
Age	> 5 years	14	335	4.14	
	< 5 years	0	16	0	
Pregnancy	Positive	2	36	5.55	p>0.05
	Negative	12	288	4.16	
Metritis	Presence	0	10	0	
	Absence	14	339	4.12	

G1-IMP: first generation of importation

Professional's serology response to *Brucella sp.*

Screening with respect to the brucellosis of 31 workmen of El-Harrach's slaughterhouse having cumulated a working mean duration of 11 ± 9.5 (range 1.5-38) years, detected a prevalence of 0%.Among workers recorded zero prevalence, 27 [87.1%] handled the pregnant alive cows or after their set with death, 14 [45.2%] consumed raw milk of unknown origin,17 [54.83%] didn't exceed the primary education level, 12 [38.7%] account for the infectious risk of the carried-on activity.The conscience level with respect to the situation is variable.Incidentally, we found6 [19.3%] knew the word brucellosis or one of its synonyms, only 4 [12.9%] workers knew the zoonoses' aspect of brucellosis and, 12 [38%] followed the medical screening examinations concerning for the professional infectious diseases.

DISCUSSION

During the period of our study, only cull cows' subpopulation was retained for variables homogeneity preoccupations according to sex, such as metritis presence and pregnancy. Thus, considering professionals contamination risk related to vaginal virulent secretions in case of pregnancy or brucellosis metritis, only pregnancies more than three months and metritis with open-cervix were taken in account. Because of the great variability in females' pre-slaughter rest time (from few seconds to a week), some of which would be withdrawn from slaughter, a sampling protocol that combines convenience and chance was applied. To avoid a sampling bias, we have chosen visits days to be in a non-cyclic rhythm. Although at eighth week, because of the cows' owners' refusal on two successive visits, a probable bias related to non-sam-

pling of 18 cows would not be eliminated. Moreover classification of cows on racial groups was done on the bases of arbitrary criteria; which do not allow a clear discrimination. Indeed, 24.5% of individuals could be indexed in none of the groups. The conception of cows' source's map via decoding earrings' marks indicates different northern Algerian wilay as covering a ray of approximately 600km around El-Harrach abattoir. Consequently, our results were compared with the results of surveys carried out in farms.

The seroprevalence of brucellosis in cows reflects a previous exposure to *Brucella* because, unlike small ruminants, vaccination for cattle is not practiced in Algeria since 1995. The present investigation carried out within the slaughterhouse of El-Harrach showed on a side a rate of 4.56 ± 0.43% of cows cull reached of brucellosis, which is definitely lower than that reported by Schoonman (2007) in a survey concerning the bovines of reform in Tanzania, where 12% of the bovines detected with the RBT were positive. On another side, 50% daily frequency appearance of an infected cow at the slaughterhouse, with a significantly higher frequency in May compared to the other months.In Algeria, Kaaboub et al. (2019) reported 12.28% and 2.5% of *Brucella* seropositivity at farm level and slaughterhouse, respectively. However, higher seroprevalences of brucellosis were observed in other studies conducted in Algeria, with values of 33.33% (Kardjadj, 2018) and 8.2% (Aggad and Boukraa, 2006). The rates of infection vary greatly from one country to another, within a country and production systems, which could be associated with the evolution of disease, geographical origin, breeds, sample size, study frame as well as the protocol adopted such as the type and number of diagnostic tests used (Awah-Ndukum et al., 2018).

Since cows reformed for brucellosis were presented to slaughterhouse without any visible mark indicating their state, which is in contrast with the Algerian legislation about medical slaughter of brucellosis cows,distinction, in our study, between cows reformed for brucellosis reason and those reformed salubrious was done by checking the slaughter certificates.Consequently, a prevalence of 4.01% was assessed in cows reformed within the healthiness framework of elimination. This rate is higher than that reported in the center area of Algeria by Lounes, (2007), at bovines subjected to systematic control in an investigation at the veterinary laboratory of Draa Ben Khedda and central laboratory of Algiers where she recorded an individual prevalence of 0.81% (0.12 to 2.82%) at the bovines, with 0.91% in females, usually screened by the RBT then confirmed by the Complement Fixation Test (CFT).This discordance can be explained by the age, the test of screening and most probably by a different zoo-sanitary situation, knowing that during the years 2010/2011, the systematic control had strongly decreased because of the lack of Rose Bengal reagent. Indeed, our sample composition, laying out 95.5% cows of more than 5 years, can constitute a variability factor of the prevalence. However, the results connecting the age to the prevalence of brucellosis are variable (Swai and Schoonman, 2010; Al-Majali et al., 2009;Delafosse et al., 2002; Kubuafor et al., 2000).Moreover, in Algeria, the mobility of the identified bovines from one owner to another is not automatically followed by the change of earrings. The situation let's suppose that breeding practices and livestock movements can be involved on these differences. On the other hand, the false positives have often limited the precise diagnosis of brucellosis and its eradication. The persistent quest for better diagnostics emphasizes the inexistence of the perfect test (easy and robust, affordable and able to identify all infected individuals) but also the fact that brucellosis is a disease in which the results and applicability of diagnostic tests are affected by technical issues and complex biological, epidemiological and socio-economic factors (Ducrotoy et al., 2016). Hence, no test gives sensitivity and a specificity of 100% (Agasthya et al., 2007). Therefore, culture is the only suitable gold standard for defining a true positive infection status in animals. Although, obtaining the high sensitivity is laborious because it requires the right culture media or a combination of media (Ducrotoy et al., 2016). Added to that, indirect methods were mostly used for the diagnosis of brucellosis, due to the lower cost of serological methods compared to PCR and microorganism isolation, as well as to the safety issues and time saving process compared to bacterial culture (Pereira et al., 2020). The RBT is known to have a raised analytical sensitivity but a low specificity compared to the other serologic methods (Khames et al.,2020). The possibility exist that a higher frequency of false positives and tests occurs when RBT is used relative to other tests which led to the recommendation that the RBT should not be used as a stand-alone test to determine the seroprevalence of brucellosis (OIE, 2009).For stage with the problems of low specificity, the confirmation of positive samples with the RBT by CFT or ELISA is essential. This confirmation is not

made in this study for logistic and laboratory reasons. Consequently, the resulting rates are adjusted on the sensitivity and the specificity of the test to reflect the true prevalence of the disease. Moreover, since vaccination against bovine brucellosis is not practiced any more in Algeria, false seroprevalence due to vaccination is discarded.In addition, in a survey performed in the District of Blida, Déchicha (2003) showed an individual prevalence at the bovine species of 7.97%; this rate is definitely higher than ours.

No difference of prevalence related to the race and to the identification statute, between the animals subjected to control and those not detected within the framework of prophylaxis plan adopted by the veterinary services was established. These results let us evaluate the effectiveness (cost/benefits) of the control measures of brucellosis in Algeria as well as the possible effects on the public health. The appearance of brucellosis at individuals is related to the prevalence in animals and to the practices which expose them to the infected animals or their products (Schoonman, 2007).The uncontrolled movements of animals as well as the introduction of animals of an unknown medical statute, even systematically screened into herds, constitute factors limiting the effectiveness of the control plan against brucellosis. As reported by Zhang et al. (2018), the subsequent rise in brucellosis prevalence was due to the difficulties in detecting the infected animals in each herd when the prevalence was extremely low. This means that animal identification and movement control have a pivotal role in the endgame when few animals are still infected. In Northern Ireland for example, a study shows that 3.1% of the bovines moved during a period of six months before the revelation of brucellosis in the herd of source, are found sero-positive in their herds of destination (Stringer et al., 2008). Whereas, what about the Algerian context? Knowing that Dechicha et al. (2003) found, in Blida, that 81.25% of the sero-positive breedings' are mixed breeding's associating several animal species in the same cattle shed.

We have assessed the prevalence in pregnant cows, because, it has shown great concentrations of *Brucella sp.* in the bovine's placenta (Lopez-Goni and Moriyon, 2005).Moreover, under conditions of high hygrometry, low temperatures and defects of natural light, *Brucella sp* can remain viable during several months in water, abortion product; feces, wools, hay, equipment and clothing (Al-Talafhah et al., 2003;Lopez-Goni and Moriyon, 2005).Considering the design and the operation of the slaughterhouse of El-Harrach, associated to the various stresses which undergo the animals, a daily frequency appearance of an infected cow of 50% (28.6 to 83.3%), the rates of 4.56% in cull cows and 5.55% in the pregnant cows handled by the near totality of the workmen, either of their alive or after their set with death, can constitute a threat for the professionals of the slaughterhouse. Indeed, in Africa, like in Algeria, human brucellosis is not well studied compared to brucellosis in the animals(Smits and Culter, 2004).

In spite of 45.2% among the tested professionals consumed in addition to the raw milk of unknown origin, the screening by RBT of 31 workers of El-Harrach's slaughterhouse having cumulated working mean duration of 11 ± 9.5 years, revealed a prevalence of 0%.This result unmatched with those reported in literature via investigations carried out in the municipality of Annaba in Algeria where a prevalence of 6.5% from agricultural professionals was noted (Tourab et al.,1990). Moreover, the reported seroprevalence of brucellosis among butchers and slaughterhouse workers in the South-Eastern Iran was estimated to 7.9% (Esmaeili et al., 2016). Group most affected by occupational brucellosis are butchers and abattoir workers, probably due to the regular manipulation of sharp objects and to close contact with potentially animals and their organs. From the slaughterhouse workers standpoint, higher contamination levels with *Brucella* were recorded in different studies. In a survey from South Korea, 62 of the 922 slaughterhouse workers (6.7%) exhibited *Brucella* seropositivity (Acharya et al., 2018).In Ngaoundéré (Cameroon), at the professional slaughterhouses, where a prevalence of 5.6 % was noted (Awah-Ndukum et al., 2018),in Bahr el Ghazal region, South Sudan, among 234 slaughterhouse workers screened by RBT and c-ELISA, 32.1% were sero-positive to brucellosis(Madut et al., 2019). Other contamination rates with brucellosis, ranging between 3.4% and 75.2%, have been observed in other countries (Awah-Ndukum et al., 2018; Zakaria et al., 2018; Kolo et al., 2019).

Among workers recorded zero prevalence, may probably be related to the defect of representativeness during sampling due to the refusal of participation with a blood test. Thus, on top of 60 existing permanent workmen, only 18 took part in the investigation. The thirteen others undergo only one occasional contact with the potentially virulent matters. Gulay et al.(2006)judged that brucellosis should be

considered among all patients presenting osteoarthritis complaints, by maintaining in the spirit the "prozone" phenomenon, before the recourse to laboratory diagnoses of point. The hazard may be decreased when prophylactic measures are properly adopted. In addition, the low educational level of abattoir workers, as well as insufficient knowledge about brucellosis, particularly on its transmission and clinical signs, increases the risk of contamination (Pereira et al., 2020).On its side,Carip et al.(2008) show that, the isolation frequency ofthe *melitensis* species borders the 80%in human pathology. However, brucellosis latency cases, going up to 70 years were also reported (Meneses et al., 2009).

Thus, brucellosis disease gravity depends, in addition to the infecting amount, of the species and even of the accused stock. Indeed, the bacillus isolation on the bovines in Algeria can bring some precise details on the brucellosis epidemiology at the slaughterhouse's workers. Furthermore, in contrast to our results, the specific investigations previously quoted were performed on volunteers coming from various establishments. Factors related tothe frequency appearance of infected animals, to functional and architectural variations of the slaughterhouses and to the undertaken measures of hygiene and safety, can plead in favor of such discordance. Finally, only systematic screening, chronologically structured, could reveal the prevalence as well as the incidence of brucellosis at the professional slaughterhouses in Algeria.In fact, abattoir facilities can be used to monitor disease control policies, detect newly introduced disease agents and to assess intervention programs, and most importantly, abattoir survey may also facilitate early intervention to mitigate the epidemic loss of animals.

In conclusion, our study showed a wide-ranging *Brucella* seropositivityin culled cows.These findings confirmed the endemicity of *Brucella* infections among cattle in Algeria. More so, the potential threat of the endemicity of animals poses to abattoir workers and other at-risk persons calls for the heightened surveillance for brucellosis among febrile patients. Educational programs and the establishment of control and prevention measures are needed to reduce the incidence of brucellosis.

CONFLICT OF INTEREST

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors declare that they have no conflict of interest.

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