Prevalence of bovine and human brucellosis in western Algeria: comparison of screening tests

H. Aggad[1] and L. Boukraa[1]

معدل انتشار داء البروسيلات في البقر والبشر في غرب الجزائر:
مقارنة بين اختبارات التحري

حبيب عقاد، العيد بوكراع

الخلاصـة: أُجريت دراسة سيرولوجية في إقليم تيارات في غرب الجزائر على 1032 بقـرة موزعـة علـى 95 قطيعـاً، بغرض تقدير معدل انتشار عدوى البروسيلات والمقارنة بين مجموعة من اختبارات التـراصّ مـن حيـث حـساسيتها ونوعيتها. وكانت اختبارات التحرِّي إيجابية في 31.5٪ من القطعان عند اسـتخدام اختبـار المستـضد المـدروء علـى الصفيحة، وإيجابية في 26.3٪ من القطعان عند استخدام اختبار وردية البنغال، وإيجابية في 15.7٪ من القطعان عند استخدام اختبار تثبيت المتمِّمة. وعند استخدام اختبار تثبيت المتمِّمة كمعيار مرجعي للاختبـارات التوكيديـة، تبـيَّن أن اختبار الريفـانول هـو أكثـر حـساسية، ولكنـه أقـل نوعيـة مـن اختبـار تـراص الأنابيـب في اكتـشاف العـدوى بالبروسيلات. وقد استُفردَتْ ثلاث من الذراري من 105 عينات دم مأخوذة من البشر المصابين بداء البروسيلات، ومن 50 عينة من اللبن والأنسجة المأخوذة من البقر المصاب بالعدوى. واتضح أن جميـع هـذه الـذراري تنتمـي إلى الضرب البيولوجي الثالث من البروسيلة المالطية.

ABSTRACT A serological study was carried out in Tiaret province in western Algeria on 1032 cows distributed in 95 flocks to estimate the prevalence of *Brucella* infection and to compare the sensitivity and specificity of a range of agglutination tests. Screening tests showed 31.5% of herds positive using the buffered plate antigen test and 26.3% using the rose Bengal test compared with 15.7% with the complement fixation test. Using the complement fixation test as the gold standard for confirmatory tests, the Rivanol test was found to be more sensitive but less specific than tube agglutination in detecting brucellosis infection. Three isolates were identified from 105 blood samples from humans with brucellosis and 50 samples of milk and tissues from infected cows and they were all *Brucella melitensis* biovar 3.

Prévalence de la brucellose humaine et bovine dans l'ouest de l'Algérie : comparaison des tests de dépistage

RÉSUMÉ Une étude sérologique a été réalisée dans la province de Tiaret dans l'ouest de l'Algérie sur 1032 vaches réparties sur 95 troupeaux pour estimer la prévalence de la brucellose et comparer la sensibilité et la spécificité des épreuves d'agglutination. Les tests de dépistage ont montré que 31,5 % des troupeaux étaient positifs en utilisant l'épreuve d'agglutination sur plaque à l'antigène tamponné et 26,3 % en utilisant l'épreuve au rose Bengal par rapport à 15,7 % avec l'épreuve de fixation du complément. En utilisant l'épreuve de fixation du complément comme méthode de référence pour les tests de confirmation, on a constaté que le test au Rivanol était plus sensible mais moins spécifique que l'épreuve d'agglutination en tube pour le dépistage de la brucellose. Trois isolats ont été identifiés dans 105 échantillons sanguins provenant de personnes atteintes de brucellose et 50 échantillons de lait et de tissus provenant de vaches infectées ; pour les trois, le biotype a été déterminé comme étant *Brucella melitensis* biovar 3.

[1]Department of Veterinary Sciences, Faculty of Agronomic and Veterinary Sciences, Tiaret University, Tiaret, Algeria (Correspondence to H. Aggad: h_aggad@yahoo.com).
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Introduction

Brucellosis is considered the most important of the zoonoses in the Mediterranean region and its economic impact is great [*1–3*]. Although steady progress is being made in brucellosis control in this region, serious difficulties remain due the complexity of the epidemiology of the disease.

An intensive government effort has been made in Algeria as well as in other North African countries to eradicate brucellosis or at least to keep the infection at a manageable level [*4*]. Data about the prevalence of brucellosis in animals are incomplete and cover only a small proportion of the national livestock. In Algeria, there has been little previous research into brucellosis. The lack of cooperation between the public health and veterinary sectors, as well as the absence of information exchange between neighbouring countries complicates the situation. A thorough evaluation of the laboratory tests currently available is also needed in order to establish the most useful tests for use in Algeria, both in humans and animals.

This investigation was carried out to study the prevalence of *Brucella* spp. infection among cattle in western Algeria, to evaluate a number of serological tests and to discuss some epidemiological aspects of brucellosis.

Methods

Background

The study was carried out between January 2002 and May 2003 in the Tiaret province of western Algeria. The study region is agropastoral with a semi-arid climate. The study animals were local cross-breeds. All investigated animals were bred in an extensive farming system and contact between the different species (cattle, sheep and goats) is not uncommon. Many diseases are endemic in the studied species (brucellosis, foot and mouth disease, tuberculosis, rabies) and the main clinical signs of several diseases are diarrhoea, abortion, lameness and respiratory problems. The breeding system is traditional without application of any standard in hygiene, food rationing or herd management. Vaccination against foot and mouth disease is available but rarely used. Vaccination against brucellosis in cattle is still forbidden in Algeria. Raw milk and milk products are widely consumed by the population.

Samples and testing

A serological study was carried out on samples of blood collected from 1032 cows with unknown pathologic status belonging to 95 herds in 3 areas of Tiaret province. The study animals were divided into 3 groups according to the area where they were found: Tiaret town, southern Tiaret and northern Tiaret. All bovine sera were subjected initially to the rose Bengal plate test (RBT) and the buffer acidified plate antigen test (BAPAT). The confirmatory tests applied were the tube agglutination test (TAT), and the Rivanol plate agglutination test (RIV). The complement fixation test was (CFT) used as the gold standard for measuring the sensitivity and specificity of the different tests.

The milk ring test (MRT) was used on a total of 765 bovine milk samples from 86 herds and 39 milk samples from slaughtered cows. The animals were cows 2 years old and over, presumed to be healthy, belonging to a designated area of Tiaret province.

A microbiology examination for isolation of *Brucella* spp. was made on 105 blood samples collected from infected humans and 50 samples of milk and tissues from infected cows. The humans (9.5% males) were patients living in Tiaret

province who had clinical and serological evidence of acute brucellosis (positive for RBT and TAT > 1/80). Of the samples from cows, 39 were from milk and 11 from tissue specimens (5 from cotyledons, 4 from lymph nodes and 2 from calf fetuses) collected from slaughtered cows with brucellosis (positive for RBT and CFT > 20 IU).

To investigate the origin of the infection in humans, 55 of the 105 patients were questioned and their responses recorded in their records.

Laboratory methods

RBT was carried out according to Alton et al. [*5*]. Equal volumes of antigen (Spinreact SA, Girona, Spain) and serum (30 µL) were placed on a plate and mixed for 4 minutes. The result was judged as positive or negative according to the presence or absence of any degree of agglutination.

BAPAT is an agglutination test in which a larger amount of antigen (80 µL) (Sentinel CH, Milan, Italy) was mixed with 30 µL of the serum under test and the plate was rotated several times and then read for agglutination [*6*].

TAT was started with a dilution of 1/10 using the European assay [*7*]. Equal volumes of antigen (Synbiotics Corp. Europe, Lyon, France) and serum (0.5 mL) were mixed. The serum under examination was diluted in order to obtain dilutions 1:10, 1:20, etc. The tubes were incubated for 20 hours at 37 °C. A sample was recorded positive when the examined tube presented agglutination and sedimentation with the clear supernatant.

RIV was done according to Alton et al. [*5*]; 0.4 ml of serum under test was mixed with equal volume of rivanol solution (Veronal buffer solution, Synbiotics Corp. Europe, Lyon, France) and centrifuged. A quantity of 30 µL of rivanol antigen (Veterinary Serum and Vaccine Research Institute, Cairo, Egypt) was added to 80 µL, 40 µL, 20 µL and 10 µL of supernatant fluid and mixed as recommended by the manufactured. Complete agglutination at 1:25 was considered positive.

CFT was done using the cold technique on micro-titres plates depending on 50% haemolysin of sheep erythrocytes [*5*]; the complement antigen (Synbiotics Corp. Europe, Lyon, France) and haemolysin were standardized and titrated before conducting the test. Each serum sample was diluted to 1:4 with barbital buffer and inactived at 56 °C for 36 min. The sensitized sheep erythrocytes were prepared by mixing equal volumes of diluted haemolysin in 3% of standardized erythrocytes solution suspension and left for 15 minutes at room temperature for sensitization. Briefly, with complete fixation, there was no lysis and a button of erythrocytes was seen at the bottom of each well and the supernatant was clear and colourless. A titre of 20 IU (1/4) or higher was considered positive. Complement and haemolysin for sensitization of sheep erythrocytes in the presence of complement was obtained from Bio-Mérieux SA, Marcy L'étoile, France. Alsever's solution for the preservation of sheep erythrocytes was purchased [*5*] and the sera were stored at –20 °C until the serological tests were performed.

The MRT was carried out according to Alton et al. [*7*]; 1 mL of milk was mixed with 30 µL of ring test antigen (Synbiotics Corp. Europe, Lyon, France) and incubated at 37 °C for 1 hour. If the intensity of blue colour in the cream layer was equal or deeper than the skim portion, the test was considered positive.

Bacteriological examination

Trypticase soy agar (TSA) plates with 5% sterile horse serum, polymyxin B (5 U/mL), bacitracin (25 U/mL) and cycloheximide

(100 µg/mL) were used for the isolation, identification and typing of *Brucella* spp. Plates were placed in an incubator at 37 °C with 10% CO_2 tension adjusted automatically.

Cultured plates were examined for *Brucella* spp. growth at the 4th day and daily for 4 weeks. Suspected colonies were further identified and subcultured on *Brucella* agar slopes. The typing of *Brucella* isolates was made according to CO_2 requirement, H_2S production, growth in the presence of dyes (thionine and basic fuchsine), reaction with monospecific sera (immunoglobulin (Ig)A and IgM) and bacteriophage typing [*5*].

Results

Screening tests

Among the studied cattle, 31.5% of herds were positive for brucellosis (1 or more animal positive) and 9.7% of individual animals were positive when using the BAPAT. The same data using the RBT were 26.3% of herds and 8.2% of individuals (Table 1).

A large difference in prevalence was seen in groups from different areas. Applying the BAPAT, prevalence among herds was 24.0%, 47.2% and 20.0% in Tiaret town, southern Tiaret and northern Tiaret respectively. The same test showed 7.8%, 11.3% and 8.2% positive among individual animals in the same areas respectively. Using the RBT, prevalence also varied greatly across the areas, especially among herds (24.0%, 36.1% and 17.6% in Tiaret town, southern Tiaret and northern Tiaret respectively).

The total prevalence of brucellosis according to the MRT (Table 1) was about 18.6% in herds and 4.0% in individuals but with a large variation between the studied areas: from 8.3% in Tiaret town to 24.1% in northern Tiaret.

Table 1 **Results of agglutination screening tests on bovine serum and milk samples from herds and individual cattle**

Area and category	Serum samples BAPAT		Serum samples RBT		Milk samples MRT	
	No. tested	% +ve	No. tested	% +ve	No. tested	% +ve
Tiaret town						
Herds[a]	25	24.0	25	24.0	24	8.3
Individuals	216	7.8	216	6.9	162	3.0
Southern Tiaret						
Herds[a]	36	47.2	36	36.1	33	21.2
Individuals	501	11.3	501	9.3	316	4.1
Northern Tiaret						
Herds[a]	34	20.5	34	17.6	29	24.1
Individuals	315	8.2	315	7.3	287	4.5
Total						
Herds[a]	95	31.5	95	26.3	86	18.6
Individuals	1032	9.7	1032	8.2	765	4.0

[a]*Positive herd = minimum of 1 animal positive to test.*
RBT = rose Bengal test; BAPAT = buffered acidified plate antigen test; MRT = milk ring test.

Confirmation tests

The results of confirmation tests indicated more positive cases using the RIV (22.1% and 3.0%) than the TAT (21.0% and 4.9%) and the CFT (15.7% and 2.6%) for herds and individuals respectively (Table 2). Applying the RIV, a large difference was seen among animals between different areas: 12.0%, 30.5% and 20.5% for herds and 2.3%, 3.7% and 2.2% in individuals respectively for the 3 above areas. With the TAT, there was also a large difference in prevalence according to each area tested, in herds (12.0%, 30.5% and 17.6%) and in individuals (3.2%, 4.5% and 6.6%). Finally, the prevalence of brucellosis according to the CFT was 8.0%, 25.0% and 11.7% for herds and 1.8%, 3.9% and 0.9% for individuals respectively.

Table 3 shows the sensitivity and specificity of the tests using CFT as the gold standard. The RBT, BAPAT and RIV tests showed 96.3%, 100% and 85.2% sensitivity respectively. BAPAT showed a higher sensitivity than the MRT but the MRT had the higher specificity (98.6%). TAT had a higher sensitivity (88.9%) than the RIV test which had a higher specificity (99.2%).

Isolates

Only 3 bacterial isolations of *Brucella* were successfully characterized: 2 isolates from the 105 human blood samples and 1 isolate from the 39 bovine milk samples. All were identified as *Brucella melitensis* biovar 3. None of the tissue or fetal samples were positive.

Source of infection

Among the 55 humans with evidence of brucellosis, the majority (49, 89.1%) had ingested raw milk or milk products and 3 (5.4%) had contracted the disease by contact with cattle or goats during parturition. The etiology of the remaining 3 (5.4%) cases was unknown.

Table 2 **Results of agglutination confirmatory tests and the complement fixation test on bovine serum samples from herds and individual cattle**

Area and category	No. tested	Serum samples RIV % +ve	TAT % +ve	CFT % +ve
Tiaret town				
Herds[a]	25	12.0	12.0	8.0
Individuals	216	2.3	3.2	1.8
Southern Tiaret				
Herds[a]	36	30.5	30.5	25.0
Individuals	501	3.7	4.5	3.9
Northern Tiaret				
Herds[a]	34	20.5	17.6	11.7
Individuals	315	2.2	6.6	0.9
Total				
Herds[a]	95	22.1	21.0	15.7
Individuals	1032	3.0	4.9	2.6

[a]*Positive herd = minimum of 1 animal positive to test.*
RIV = Rivanol test; TAT = tube agglutination test; CFT = complement fixation test (gold standard).

Table 3 **Inter-test comparison between different tests for brucellosis and the complement fixation test as gold standard**

Test	CFT No. +ve	CFT No. –ve	Sensitivity %	Specificity %
RBT			96.3	94.1
+ve	26	59		
–ve	1	946		
BAPAT			100.0	93.7
+ve	27	63		
–ve	0	932		
MRT			87.5	98.6
+ve	21	10		
–ve	3	731		
TAT			88.9	97.3
+ve	24	27		
–ve	3	978		
RIV			85.2	99.2
+ve	23	8		
–ve	4	997		

RBT = rose Bengal test; BAPAT = buffered acidified plate antigen test; MRT = milk ring test; RIV = Rivanol test; TAT = tube agglutination test; CFT = complement fixation test (gold standard).

Discussion

The recorded incidence of brucellosis has increased in Algeria during the last few years. This may be a recording artefact due to the availability of improved diagnostic tools but it is also likely to be a real effect resulting from importation of cattle. Brucellosis is widely regarded as an insidious disease, demanding the most thorough care in diagnosis. No single test is capable of identifying all positive cases [*8*].

We can explain the high prevalence of brucellosis in this study by the fact that the control of brucellosis is insufficient in Algeria; indeed only a small proportion of the cattle are controlled. The prevalence of brucellosis for individual animals (2.6% using the CFT) was higher than that declared by the Algerian department of veterinary services [*9*]. This authority recorded a percentage of positive cases varying between 0.6% and 1.5% from 2001 to 2002 with tests on only 6000 to 8000 cattle, less than 11.5% of the total national dairy bovine population.

The prevalence of the different species of *Brucella* differed greatly between geographical regions. The differences depend on the fact that the breeders do not respect the international norms in conducting their herds: indeed the mean number of animals per herd was 11 and several visited herds comprised 2 to 5 animals. Ignorance of farmers, lack of hygiene and moving animals without any control procedures are probably contributing to the spread of the infection. We have also noted that the majority of infected animals, including those

tested and slaughtered according to the state brucellosis eradication programme, were of imported breeds.

The ideal standard test that confirms the disease is the isolation of the bacterial agent [*10*]. To our knowledge, isolation of *Brucella* spp. has not been reported before in Algeria and we continue to investigate with serological methods on both human and animal samples. However, animals with low serological titres missed during the testing can play a great role in spreading the infection.

Since the principal source of infection in humans was reported to be consumption of unpasteurized cow's milk and milk products, the isolation of *Brucella melitensis* biovar 3 from human blood and from bovine milk means that these bacteria have probably passed from small ruminants to bovines then to consumers. This is the predominant strain among infected animals and humans in most Mediterranean countries [*11*]. The great majority of animal tissues investigated in our study were negative, due to the fact that in chronic brucellosis, microbiological studies are usually negative [*12*] as these affected animals have already received antimicrobial treatment. Such a situation is very serious in the absence of any control in the sale and administration of veterinary medicines.

It is evident that more investigations are needed in all provinces of Algeria in order to obtain full information about the epidemiology of brucellosis in animals and humans. However, the high levels of cattle importation may increase the incidence of *Brucella* and bias such investigations. In order to prevent the introduction of new species of *Brucella*, strict controls must be applied to animal importation.

The majority (89%) of humans infected with brucellosis had ingested raw milk or milk products, mostly in the spring and summer seasons that coincide with the parturition and lactation period of cattle and goats. Another study showed the role of milk in 85% of human cases in Algeria [*13*]. In 2002, 250 people living in Tiaret province were treated for brucellosis [*14*]; the main reason for contracting the disease was consumption of raw milk and milk products. Among them, 159 were from the region of Frenda located in southern Tiaret, an area which in our study had high percentage prevalence. Milk and milk products have been reported as the main factor in brucellosis infection in humans in several other countries: 70.4% in Palestine [*15*] and 83% in Kuwait [*16*].

Comparing the screening tests—BAPAT, RBT and MRT—our results showed a higher sensitivity using BAPAT to that recorded in Egypt [*17*] and we can recommend BAPAT be introduced in programmes of brucellosis control in Algeria, because it is more sensitive as a screening test than the RBT. Buffered antigen tests are suitable for screening herd and individual animals [*18*]. The higher sensitivity of BAPAT compared with other serological tests can be attributed to the final pH of the test (4.02) which enables some of the anti-*Brucella* IgM beside IgG, IgG1, IgG2 and IgA to share in the reaction [*19*].

On the other hand, the relatively higher acidic pH of RBT (3.65) allows smaller amounts of IgM to share in the reaction, since this class of immunoglobulin is known to be acid-labile [*20*]. This pH enhances the specificity of the RBT. RBT does not need special laboratory facilities and is simple and easy to perform. The test detects specific *Brucella* antibodies of the IgM and IgG types and is more effective in detecting antibodies of the IgG1 type than IgM and IgG2 types. However, the temperature of the antigen and the ambient temperature

may influence the sensitivity and the specificity of RBT [*21*].

MRT was more specific in brucellosis screening than the RBT and BAPAT tests and can replace them. MRT is cheap, easy, simple and quick to perform. It detects lacteal anti-*Brucella* IgM and IgA bound to milk fat globules. The use of this test, however, is limited by the milk quality. It can test false positive when the milk contains colostrum, at the end of the lactation period and in cows with mastitis [*22*]. Milk with low concentrations of lacteal IgM and IgA, or lacking the fat-clustering factors, can test false negative [*23*].

The confirmatory agglutination tests were assessed using positive results in the CFT as the gold standard. The study revealed a lower sensitivity of RIV compared with RBT and BAPAT. This can be explained by the fact that RIV precipitates IgM [*24*], which appears later in the infection and is not revealed by RIV, since it detects only the IgG. Thus, RIV cannot detect animals in the early stage of infection and cannot replace CFT. The sensitivity and specificity of TAT were 88.9% and 97.3% respectively and the sensitivity and specificity of RIV were 85.2% and 99.2% respectively. Compared with RIV and TAT, which showed nearly the same prevalence of *Brucella* spp. (22.1% and 21.0%) for flocks but not for individuals (3.0% and 4.9%), it appears that TAT was a little more sensitive. This fits with the data showing that TAT is an IgM detector [*25*] and CFT principally an IgG1 detector [*26*]. TAT is relatively simple and easy to perform and requires basic laboratory equipment. It can be used to detect acute infections, as antibodies of the IgM type usually appear first after the start of the infection and are more reactive in TAT than antibodies of the IgG1 and IgG2 types. However, it can give false negative or false positive results [27].

CFT is considered the best confirmatory test for the diagnostic of brucellosis. When the disease becomes chronic, the titres detected by TAT tend to be negative, whereas titres detected by CFT remain at perceptible levels [*28*]. CFT is laborious and requires highly trained personnel as well as suitable laboratory facilities. Although its high specificity is very important for the control and eradication of brucellosis, it may test false negative when antibodies of the IgG2 type hinder complement fixation [*21*]. CFT measures more antibodies of the IgG1 type than antibodies of the IgM type, as the latter are partially destroyed during inactivation.

In Algeria, the serological tests used in bovine brucellosis control are RBT and CFT, which seems to be acceptable. This requires that the serological testing is carried out periodically in herds, after the slaughtering of infected animals, in order to detect any increase in the antibody levels of animals with suspicious titres.

Conclusion

As an endemic country, Algeria must consider the epidemiological surveillance of human and animal brucellosis as a priority. This can be achieved by performing, regularly and adequately, more sensitive screening tests and by cooperation between public health and veterinary sectors as well as information exchange between neighbouring countries. Due to the presence of the disease in complicated phases in different livestock, and because different management systems are applied in animal breeding, none of the serologic tests alone can identify all positive reactors. Therefore, in developing countries it is advised that more than one screening test should be applied throughout the implementation of a surveillance programme, with the CFT as a confirmative test.

The study confirms the presence of *Brucella* species among humans in Algeria and shows a sizeable prevalence rate among cattle. Thus, farmers need to be informed about the risks of cross-infection between goats, sheep and cattle, and ultimately humans.

Instead of adopting eradication as the sole policy, the Algerian government is soon to experiment with vaccination in small ruminants with *Brucella melitensis* Rev 1 strain, starting in 4 provinces. Although vaccination could interfere with the surveillance programme [*29*], several approaches have been developed to overcome this problem. The common approach is to vaccinate only the female lambs and young goats between the ages of 2 to 6 months and exclude them from the serological tests until their vaccine titres disappear.

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