

Brucellosis in the West of Algeria

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Abstract

Brucellosis, the most common bacterial zoonosis in both human and animals, has a widespread geographic distribution. To prevent this disease in cattle (in the western region of Algeria) and to preserve the quality of the milk and other derivatives, we screened for detecting the presence of serum antibodies (against *Brucella*) by different immunochemical tests. The wilayets (state) concerned were from Mascara, Relizane, Tiaret and Tissemsilt. The study involved the involvement of techniques such as the buffered antigen test (EAT), indirect enzyme immunoassay (i-ELISA) and the complement fixation (CF). 744 cattle were involved for this investigation. In the wilaya of Mascara, 418 cows were investigated of which only 2 cases were found to be positive by using the EAT and 99 test cases were tested using ELISA. At Tiaret, the total number of dairy cows investigated were 156 out of which, only 1 case was held by EAT positive but the use of immunoassay test showed 14 positive results. A similar observation was made for around 170 cows which were tested in Relizane province where, 8 cases were positively tested using ELISA and the other tests were found to be negative. Out of 5 cows on Tissemsilt controlled, only 1 positive case was detected by ELISA.

The results derived using these three tests identified performance of the immunoassay where many cases of brucellosis found negative in the test by the use of the buffered antigen test and the fixing of supplement. The ELISA was diagnosed with better sensitivity, as among the 744 sera tested, only 3 sera were found positive by the use of tests of the EAT and FC but in case of ELISA, 112 cases were detected positive. The animals which reacted positively towards the ELISA had not done any screening using EAT. At a prevalence of 15.05%, this disease (zoonosis) exists in western Algeria which was revealed by screening of the cattle using ELISA test by which the sensitivity and performance were recognized.

Keywords: Brucellosis; Dairy cows; EAT; ELISA; FC

Introduction

Brucellosis, the most common bacterial zoonosis in both human and animals, has a widespread geographic distribution [1]. Worldwide, approximately 500,000 new human cases of brucellosis are reported annually [2]. Although brucellosis is endemic in many parts of the world, especially in Mediterranean countries, north and east Africa, the Middle East, central Asia and Latin America, this disease often goes unrecognized or unreported.

Brucellosis is a medical condition which is caused by small Gram negative cocco-bacilli of genus *Brucella*.

In 1887, Sir David Bruce (a British military physician) was the first to isolate this causative agent from the spleen of patients who died from Mediterranean fever in Malta [3]. The genus *Brucella* is negative gram [4], consists of seven species, including four that are pathogenic to humans: *B. melitensis*, *B. abortus*, *B. suis*, and *B. canis*. In low and middle income countries the most common mode of acquiring human brucellosis is through the consumption of contaminated milk or dairy products. Other modes of transmission are through contact and inhalation of organisms from infected animals, principally cattle, goats

and sheep [5]. *Brucella* organisms may persist for 5-15 days in milk, 30 days in ice cream, 142 days in butter, and for several weeks in tap water [6,7].

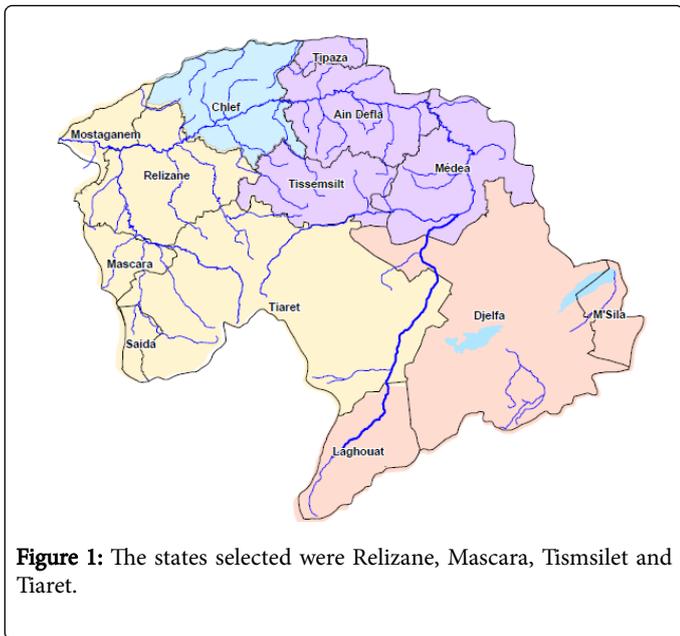
The economic impact of brucellosis in cattle can be enormous by affecting only the breeding animals along with associated losses due to unexpected deaths, still births, abortion and insufficient milk production. In case of humans, the disturbance in reproduction or infertility is far beyond any economic stress, because it can hardly be analyzed in medical care [8].

Materials and Methods

Study design

From February to June 2009, a cross-sectional study was performed out at in west of Algeria (Mascara, Relizane, Tiaret and Tissemsilt) to check the sero-prevalence of bovine brucellosis. 744 cattles were involved for this investigation.

The states selected were Relizane, Mascara, Tisimsilet and Tiaret (Figure 1).



Sample collection

Blood Sample: Blood was collected from the coccygeal, jugular or saphenous veins into the vacuum container tubes, which were then instantly placed in an ice bath and transported to the laboratory within 7 hours. When the outside ambient temperature (environment) was cool, the clot was allowed to form in the vacuum container tube at the field before transportation. The samples were then centrifuged at 3,000 rpm for 15 minutes and the serum was removed and stored at -20°C until analysis was done.

Laboratory analysis

Serological tests were performed to analyze the brucellosis [9].

Screening using Rose-Bengal plate-agglutination test

The laboratory based screening was performed using Rose Bengal Plate Test (RBPT) depending on agglutination of antigen (colored particulate) (killed *Brucella* organisms) by the antibodies (Ab) present in the cattle's serum) along with complement fixation test (CFT) [10].

Competitive enzyme-linked immunosorbent assay

The test was performed using c-ELISA kit (from COMPELISA, VLA, Weybridge, UK.) with accordance to the manufacturer's instructions, in order to confirm the RBPT positive and in conclusive samples. The optical density (OD) was measured at 450 nm using micro-plate ELISA reader (from SIGMA DIAGNOSTICS EIA Multiwell Reader II).

A positive/negative cut-off was calculated as 60% of the mean OD of the 4 conjugate control wells. The test sample with an OD equal to or below that value was considered as positive [11].

Results

744 dairy cows were found to be affected by these different tests. In the Wilaya of Mascara, a total of 418 cows which, only 2 cases were positive by using the EAT and 99 test cases using ELISA (Figure 2).

At Tiaret the total number of dairy cows monitored is 156, of which only 1 case was held by EAT positive but the use of immunoassay test showed 14 (Figure 3).

A similar observation was made for the 170 cows tested in Relizane province. In this region 8 cases were positive using ELISA. The other tests were negative.

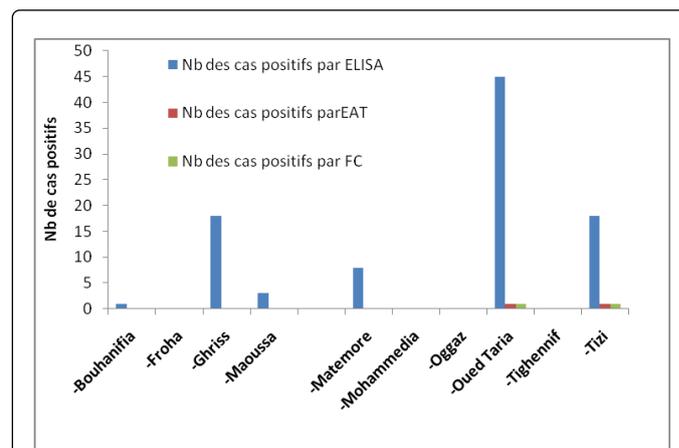


Figure 2: Rate of seropositive cows by the three tests in Mascara wilaya.

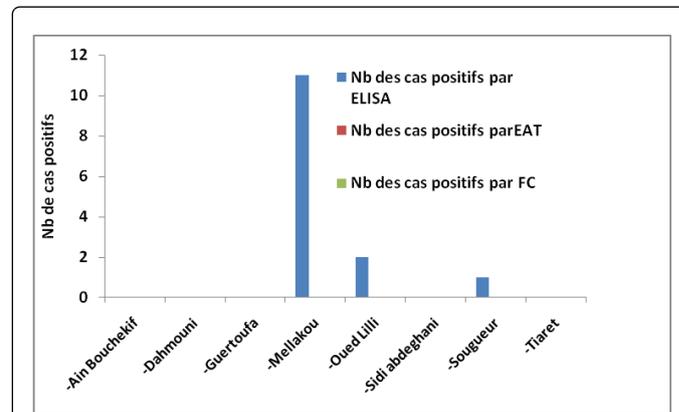


Figure 3: Rate of seropositive cows by the three tests in Tiaret wilaya.

About 5 cows on Tissemsilt controlled only 01 positive case is obtained by ELISA. The results obtained using the three tests show the performance of the immunoassay has identified many cases of brucellosis found negative in the test use of the buffered antigen test and the fixing of supplement (Figure 4).

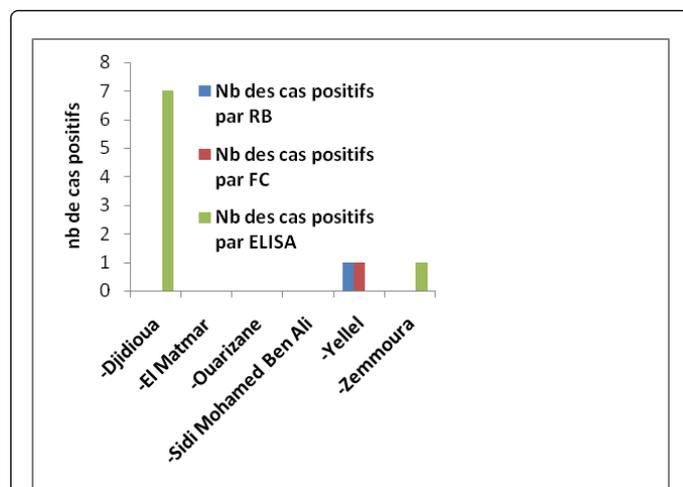


Figure 4: Rate of seropositive cows by the three tests in Relizane wilaya.

The ELISA has better sensitivity because among the 744 sera tested only 3 sera were found positive by the use of tests of the EAT and FC but 112 cases were detected positive by ELISA. The animals that reacted positively to the ELISA have not done any testing EAT while all animals tested positively are for the ELISA. This zoonosis exists in western Algeria; with a prevalence of 15.05% revealed by use of ELISA test with sensitivity and performance are recognized (Table 1).

Reaction	Rose Bengal		Complement fixation test		ELISA	
	Number	%	Number	%	Number	%
Negative	741	99.6	-	-	624	83.87
Doubtful	0	0	-	-	8	1.08
Positive	3	0.4	3	0.4	112	15.05
Total	744	100	3	0.4	744	100

Table 1: Comparison of the serological scores of the three tests used.

Discussion

In many countries, brucellosis is a notifiable disease but official figures do not fully show the number of cases which are reported annually, and the true case has been identified to be between 10 to 25 times higher than the reported figures [12].

EAT and FC have a similar sensitivity and specificity and the ELISA is significantly more sensitive for the diagnosis of brucellosis because

this test can detect up to 0.001 to 0.01 µg/ml of antibody. It is fast, easy to undertake, automatable, detecting both recent and chronic infections and allows to test a large number of sera in such a short time compared to conventional tests such as the EAT and the FC. Although the Rose Bengal tests are fast, it has many false-negative results in its chronic form [13].

Conclusion

This zoonotic infection became a serious problem for livestock and a constant threat to human health. To our knowledge, few published reports have studied the epidemiology and clinical features of this endemic disease in the west of Algeria.

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