Serological Survey of Bovine Brucellosis in Cameroon

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Keywords

Cattle – *Brucella* – Brucellosis – ELISA – Immunoenzyme techniques – Complement fixation test – Agglutination test – Morbidity – Cameroon.

Summary

A serological survey was carried out at the abattoir of Dschang (West Cameroon) from August 2002 to July 2003 in order to determine the prevalence of bovine brucellosis. Eight hundred and forty sera of cattle were examined for brucellosis by indirect ELISA (iELISA) and the rose bengal test (RBT). The seroprevalence of brucellosis was 9.64 and 4.88% by iELISA and RBT, respectively. Eighty-one samples that gave positive results in iELISA and 50 randomly selected samples that reacted negatively in iELISA and RBT were further tested by the complement fixation test (CFT) and the slow agglutination of Wright with EDTA (SAW-EDTA). All the RBT/iELISA negative samples were confirmed as negative by the other tests, suggesting that iELISA and RBT showed a high specificity in the tested population. Of the iELISA positive samples, 37.8% were classified as positive by SAW-EDTA, RBT, and CFT, 39.2% were classified as negative by SAW-EDTA, RBT, and CFT, and 23.0% were classified as positive by one or two of the three confirmatory tests. Given the lack of sensitivity of these confirmatory tests, particularly when chronicity of the infection and extensive husbandry systems (pastoralism) prevail, the best estimation of the actual prevalence of brucellosis was based on the iELISA results and was close to 10%.

■ INTRODUCTION

Brucellosis caused by bacteria of the genus *Brucella* is considered one of the most widespread zoonoses in the world (8, 15). The importance of this highly contagious disease is due both to its economic impact on the animal industry and to the severe hazard it represents to human health (17). Brucellosis caused by *B. abortus* is of serious economic importance to the cattle industry as shown by the enormous financial losses reported in several countries (10, 13, 16, 23, 26). While the disease is being eradicated in several countries of the European Union (12), it continues to be a major public and animal health problem in many regions of the world, particularly where livestock is a major source of food and income (8). In sub-Saharan Africa, brucellosis is regarded as a major problem among ruminants (13, 29), and its epidemiology and impact have been reported in several countries (15).

In Cameroon, however, no recent data is available on brucellosis. In the 1980s, a number of studies were carried out in the northern part of the country, where the seroprevalence ranged from 7.5 to 31% depending on the geographic region, using sampling methods and diagnostic techniques (3, 4, 5, 6, 7). No outbreaks of bovine brucellosis have been reported to the World Organisation for Animal Health (OIE) by Cameroon since 1996 (20). Also, the prevalence data of brucellosis from slaughter records and cattle farms are not often available or are likely to underestimate the actual prevalence

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of the disease. However, there have been case reports of brucellosis in humans from 2000 to 2002, but the most likely source was not further specified (20). Given the fact that the threat to livestock production and human health caused by *B. abortus* in Cameroon is not well known, a serological survey was undertaken at the abattoir of Dschang (West Cameroon) to assess the seroprevalence of bovine brucellosis using different serological tests.

■ MATERIALS AND METHODS

Study site

Dschang is the capital of Menoua division in the West province of Cameroon (Figure 1). The town is located between latitude 5° 27' N and longitude 10° 02' E. Mean annual rainfall ranges from 1500 to 2000 mm. The cattle population is estimated at 5250 head (MINEPIA-Dschang, 2003, pers. commun.). The majority of cattle and small ruminants are kept under an extensive animal husbandry system and are often grazing together. There is only one slaughterhouse in Dschang, where pigs and cattle are slaughtered on a daily basis. Cattle slaughtered there originate partly from local farms but the majority comes from large herds in neighboring North West and Adamawa provinces. Currently, vaccination against brucellosis in livestock is not carried out in these regions.

Data collection

Sample collection from the abattoir of Dschang took place between August 2002 and July 2003. Blood samples were collected from 840 animals, representing about two thirds of the total number of cattle slaughtered at the abattoir for a period of one year. The number of samples collected per month ranged from 12 to 142 with an average of 70 samples per month. The majority of the animals (n = 551) were females, against 289 males. All animals were zebus (*Bos indicus*) of the breed Ako (White Fulani) and Djafoun (Red Fulani), numbering 232 and 608, respectively. The ages of the animals sampled ranged from 2 to 9 years. The sera obtained were stored at a temperature of –20°C and later shipped to the Veterinary and Agrochemical Research Centre (VAR, officially accredited for brucellosis serology, Brussels, Belgium) for further analysis. During the survey, the origin of the animals was also recorded.

Serology

All 840 samples were tested by the rose bengal test (RBT) and by indirect enzyme-linked immunosorbent assay (iELISA). RBT was carried out in the parasitology laboratory of the University of Dschang (Cameroon) and results were confirmed at VAR. ELISA, the complement fixation test (CFT), and slow agglutination of Wright- (SAW-)EDTA were conducted at VAR. Only samples that gave positive results for ELISA were further tested by CFT and SAW-EDTA. Fifty samples that tested negative by both ELISA and RBT were randomly selected and tested by CFT and SAW-EDTA.

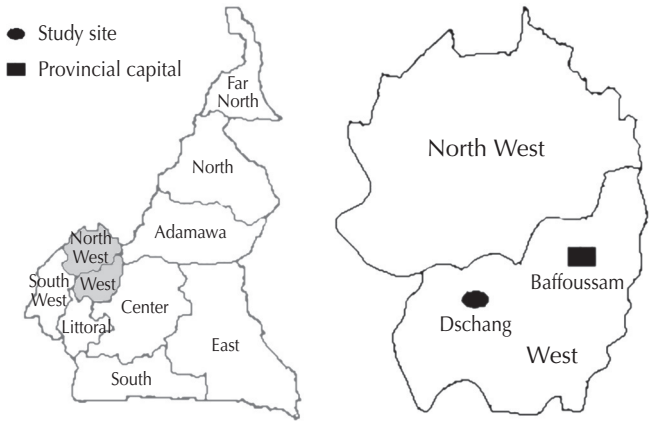

Figure 1: *Study area in the Western Highlands of Cameroon.*

Rose bengal test

RBT was performed as described by Alton et al. (2). Briefly, the sera and antigen were brought to room temperature for 45 min before use. One *Brucella* positive and one negative reference samples were used on each plate. Equal volumes (30 µl) of serum and antigen (concentrated suspension of *B. abortus*, Weybridge strain 99; Institut Pourquier, France) were mixed and rotated on a glass plate for 4 min. Agglutination values were recorded as negative (–) and positive (+, + +, + + +, and + + + +) representing different degrees of agglutination.

Indirect enzyme-linked immunosorbent assay

iELISA was performed according to Limet et al. (14) using *B. abortus* biotype 1 (Weybridge 99) as antigen. Protein G-horseradish peroxidase (G-HRP) was used as conjugate as described by Saegerman et al. (24). For the standard curve, 6 dilutions (1/1000–1/32000) of the positive reference serum (No. 1121) were prepared. Reading of optical densities (OD) was done at 492 nm and 620 nm using an automatic ELISA reader (WALLAC). The results ($OD_{492} - OD_{620}$) were expressed as antibody units in comparison with a reference serum. The conversion of ODs into units (U/ml) was done using six dilutions of the reference serum to establish a standard curve. The cut-off value was defined at 2 U/ml of test serum.

Complement fixation test

CFT was performed in microplates according to OIE's manual (19). Briefly, in 96-well microtiter plates, a 25 µl aliquot of each serum and controls (negative and positive) were serially diluted in veronal-saline buffer. A 25 µl volume of previously titrated antigen (*B. abortus* biotype 1, Weybridge 99; Antifix, Synbiotics Europe, France) was then added to each well, followed by 25 µl of complement (Virion Medical Microbiology, Switzerland). After incubation at 37°C for 30 min, 25 µl of sensitized sheep erythrocytes were added to each well and plates were again incubated at 37°C for 30 min.

After incubation, the plates were spun (500 *g* for 3 min) and the results evaluated as follows: 100% hemolysis was considered a negative reaction, while reactions showing 75, 50 or 25% of hemolysis were considered positive. Sera with positive fixations at a titer equivalent to or higher than 20 international complement fixation units (ICFTU), as prescribed by the European Union, were considered to be positive.

Slow agglutination of Wright with EDTA

SAW was carried out with EDTA as described by Garin et al. (9). The antigen used was *B. abortus* biotype 1 Weybridge 99 (Synbiotics Europe, France). Sera were serially diluted at 1/12.5, 1/25, 1/50, 1/100, 1/200, 1/400 in 96-well microtiter plates. The plates were agitated and incubated at 37°C for 20-24 h. Reading was done on the basis of the degree of agglutination and expressed in international units (IU). Any serum with an antibody titer greater than or equal to 30 IU/ml, as prescribed by the EU, was considered positive.

■ RESULTS

Seroprevalence of bovine brucellosis

Out of 840 sera, 9.64% and 4.88% were positive by iELISA and RBT, respectively (Table I). All samples which gave a positive

result in RBT were also positive in iELISA. Based on a sensitivity of 96.25% (18) and a specificity of 97.73% (24) of iELISA, the prevalence was estimated to be close to 10%, and the positive and negative predictive values of iELISA were 81.90 and 99.59%, respectively.

Over 80% of the cattle slaughtered in the abattoir of Dschang originated from the North West and Adamawa provinces, which are among the leading cattle producing regions in Cameroon. Abattoir records of the abattoir of Dschang did not reveal any information on the occurrence of brucellosis.

Evaluation of the correlation between iELISA, RBT, CFT, and SAW-EDTA

All iELISA positive (n = 81) and 50 randomly selected samples, which were negative in iELISA and RBT, were examined by SAW-EDTA, RBT and CFT. Out of the iELISA positive samples, 37.8% (28/74) were classified as positive by SAW-EDTA, RBT and CFT, 39.2% (29/74) were classified as negative by SAW-EDTA, RBT and CFT, and 23.0% (18/74) were classified as positive by one or two of the three confirmatory tests (Table II).

Table I

Seroprevalence of brucellosis in slaughter cattle at the abattoir of Dschang, West Cameroon

Test used	Num. tested	Num. positive	Seroprevalence (%)
iELISA	840	81	9.64
Rose bengal test	840	41	4.88

iELISA = indirect enzyme-linked immunosorbent assay

Table II

Comparison of the results of iELISA, RBT, CFT and SAW-EDTA in 124* samples from the abattoir of Dschang, West Cameroon

iELISA	SAW-EDTA	CFT	RBT	Total num. of cattle
0	0	0	0	50
1	0	0	0	29
1	0	0	1	4
1	0	1	1	2
1	1	0	0	3
1	1	0	1	6
1	1	1	0	2
1	1	1	1	28

* Only 74 out of 81 iELISA positive sera were available in sufficient amounts

iELISA = indirect enzyme linked-immunosorbent assay; SAW-EDTA = slow agglutination of Wright ; RBT = rose bengal test; CFT = complement fixation test

1= positive test result; 0 = negative test result

■ DISCUSSION

The seroprevalence of bovine brucellosis in the abattoir of Dschang (4.88 and 9.64 using RBT and iELISA, respectively) indicated that in the Western Highlands of Cameroon the infection was enzootic. The fact that 80% of the cattle slaughtered in the abattoir of Dschang came from the Northwest and Adamawa provinces confirmed that not only the Western Highlands were enzootic but also the Adamawa, where brucellosis had been reported previously (3). Since vaccination against brucellosis was not implemented in the region, the seroprevalence figures obtained are a reliable estimate of exposure to wild type *Brucella* spp.

There was, however, no further investigation to identify the *Brucella* species infecting cattle in this area, where breeding of cattle alongside goats and sheep is a common practice. It is therefore not possible from the results of this study to rule out that besides *B. abortus* infections, *B. melitensis*, originating from the small ruminant reservoir, may also infect cattle as described previously in the Mediterranean Basin (19, 28). This study has revealed that, in spite of the fact that official data from Cameroon about brucellosis have been lacking since 1996 (20), the disease is still enzootic in the country and the risk posed to the human population and the economy of cattle production should not be underestimated (11).

iELISA is known to be more sensitive than the traditional tests (RBT, CFT, SAW and SAW-EDTA) (19). The fact that all RBT positive samples were also classified as positive by iELISA strongly suggests that seropositivity was indeed due to sensitization by *Brucella* spp., and most probably by *Brucella abortus*. It is widely accepted that agglutination tests (SAW-EDTA and to a lesser extent RBT) are not recommended for the diagnosis of chronic brucellosis since these tests mainly detect IgM. The amount of IgM found in the sera will decline with time and become undetectable in agglutination tests in most chronic cases (19). However, in experimental conditions agglutination tests are able to detect infections as early as two weeks postinfection and thus remain excellent tools to use in order to detect early infections (12). It is also documented that CFT may not detect animals that have been recently infected naturally, or experimentally with 10^7 CFU via the conjunctival route (a dose that is known to induce 70% of abortions under experimental conditions; 21).

Altogether, the present results reinforced the fact that one needs to interpret serological results according to the epidemiological situation. In this particular case, results suggested that brucellosis was enzootic in this extensive animal husbandry system (pastoralism). In such a system, in the absence of any control program, prevalence rates and infectious loads are *a priori* low. The results suggested that, although RBT could be used as a screening test for brucellosis due to its low cost and easy execution, iELISA provided better estimates of the actual prevalence of the infection. Indeed, in this husbandry system, it was likely that the iELISA positive results were for the majority true positive results although it could not be ruled out that there might have been some false positive results due to cross-reactive bacteria or illegal use of B19 vaccination (24). The presence of *Yersinia enterocolitica* serovar O:9, that can induce false positive reactions in brucellosis serological tests, is not known in Cameroon. However, because these bacteria mainly occur in temperate regions and only induce short term serological reactions in infected cattle, it is unlikely that *Y. enterocolitica* O:9 had an influence on the prevalence rate of bovine brucellosis reported in this study. In order to validate the iELISA prevalence estimates, the use of the brucellosis skin test could be recommended given its high specificity (99.83%) (25). Lastly, a brucellosis serological survey should be conducted in small ruminants in order to assess whether,

apart from *B. abortus*, *B. melitensis* is present and hence may also infect cattle.

It was also observed that Fulani herder families in the study area consumed quite a lot of raw milk. Consequently, the risk of transmission of brucellosis to the Fulani community is a reality as was observed also among the pastoral community in Chad (11, 27).

From this study, it can be concluded that brucellosis is enzootic in the Western Highlands and the Adamawa. The risk for the human population is undisputable given the fast growing dairy farming sector and intensification of livestock production in this region of the country.

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Résumé

Shey-Njila O., Daouda, Nya E., Zoli P.A., Walravens K., Godfroid J., Geerts S. Enquête sérologique de la brucellose bovine au Cameroun

A partir d'août 2002 jusqu'à juillet 2003, une enquête sérologique a été conduite à l'abattoir de Dschang (Ouest Cameroun) afin de déterminer la prévalence de la brucellose bovine. Huit cent quarante sérums bovins ont été examinés par l'Elisa indirect (iElisa) et le test au rose bengale (TRB). La séroprévalence de la brucellose a été de 9,64 et 4,88 p. 100 en utilisant respectivement l'iElisa et le TRB. Quatre-vingt et un sérums positifs avec iElisa et 50 sérums sélectionnés aléatoirement parmi les sérums qui ont été négatifs en iElisa et TRB ont été examinés par le test de fixation du complément (TFC) et le test d'agglutination lente de Wright avec Edta (SAW-Edta). Tous les échantillons négatifs en TRB/iElisa ont été confirmés comme étant négatifs par les autres tests, suggérant que le TRB et l'iElisa montraient une haute spécificité dans la population testée. Parmi les sérums iElisa positifs, 37,8 p. 100 ont été classés positifs par SAW-Edta, TRB et TFC, 39,2 p. 100 ont été classés négatifs par SAW-Edta, TRB et TFC, et 23,0 p. 100 ont été classés positifs dans un ou deux des trois tests de confirmation. Etant donné le manque de sensibilité des tests de confirmation, en particulier lors d'infections chroniques dans des systèmes d'élevage extensifs (pastoralisme), la meilleure estimation de la prévalence réelle de la brucellose a été basée sur les résultats en iElisa et a été de l'ordre de 10 p. 100.

Mots-clés : Bovin – *Brucella* – Brucellose – Test Elisa – Technique immunoenzymatique – Réaction de fixation du complément – Réaction d'agglutination – Morbidité – Cameroun.

Resumen

Shey-Njila O., Daouda, Nya E., Zoli P.A., Walravens K., Godfroid J., Geerts S. Encuesta serológica de la brucelosis bovina en Camerún

Una encuesta serológica se llevó a cabo en el matadero de Dschang (Camerún del Oeste), entre agosto 2002 y julio 2003, con el fin de determinar la prevalencia de la brucelosis bovina. Ochocientos cuarenta sueros bovinos fueron examinados para brucelosis por ELISA indirecto (iELISA) y el test de la rosa de bengala (TRB). La seroprevalencia de la brucelosis fue de 9,64 y 4,88% mediante iELISA y TRB, respectivamente. Ochenta y un muestras que dieron resultados positivos para el iELISA y 50 muestras seleccionadas al azar que reaccionaron negativamente para el iELISA y TRB fueron examinadas luego por el test de fijación de complemento (TFC) y la aglutinación lenta de Wright con EDTA (SAW-EDTA). Todas las muestras negativas TRB/iELISA fueron confirmadas como negativas mediante otros tests, sugiriendo que el iELISA y el TRB mostraron una alta especificidad en la población examinada. De las muestras iELISA positivas, 37,8% fueron clasificadas como positivas por el SAW-EDTA, TRB y TFC, 39,2% fueron clasificadas como negativas por SAW-EDTA, TRB y TFC, y 23,0% fueron clasificadas positivas por uno o dos de los tres tests de confirmación. Dada la falta de sensitividad de los tests confirmatorios, particularmente en caso de infección crónica y sistemas de cría extensivos (pastoralismo), la mejor estimación de la prevalencia actual de la brucelosis se basó en los resultados del iELISA y estuvo cercana a 10%.

Palabras clave: Ganado bovino – *Brucella* – Brucelosis – ELISA – Técnica inmunoenzimática – Prueba de fijación del complemento – Reacción de aglutinación – Morbosidad – Camerún.