Experiences with a strategy to investigate bovine brucellosis in a rural area in Southwest Uganda

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■ PATHOLOGIE INFECTIEUSE

Key words

Cattle - Sheep - Goat - *Brucella abortus* - Brucellosis - Agglutination test - Complement fixation test - Morbidity - Uganda.

Summary

Forty-three dairy herds comprising 1094 cattle, 22 sheep and 102 goats were enrolled in a bovine brucellosis cross-sectional study. As an initial herd screening test the milk ring test conducted with bulk milk samples was chosen. Ten percent of the herds were MRT positive. Due to a low percentage of lactating cows within the herds (on average 25%), false positivity and spoilage of milk samples, MRT was found to be unsatisfactory in detecting infected herds. Therefore, 756 cattle, 22 sheep and 79 goats were screened individually by the Rose Bengal plate test (RBPT) and subsequently subjected to the complement fixation test (CFT). The agreement of RBPT and CFT was determined as moderate in this study. Based on CFT results the *Brucella abortus* overall prevalence rate was then 3% (23/756). Fifty-two percent of the seropositive cows were older than 6 years. Seropositive animals were five times more likely to previously have had an observed abortion than seronegative animals. The herd prevalence rate was calculated as 25% (10/40) and the prevalence rate within the herd ranged from 1.5 to 20.4% with a mean of 5.6%. All service bulls, goats and sheep were seronegative and thus played no apparent role in the transmission of brucellosis in the area. The test-systems applied (MRT, RBPT and CFT) are discussed and recommendations for further bovine brucellosis investigations are given.

■ INTRODUCTION

In developing countries increased intensification of cattle raising and milk production enhances problems caused by brucellosis. As the disease often goes undetected the identification of infected herds and animals is of prime importance.

In tropical Africa up to 40% *Brucella abortus* seroprevalence rates are reported among the cattle population, and in some areas these rates are even higher (4). However, in the central and southern regions of Uganda 13.6% of the cattle population is infected with *B. abortus* (3). Data on the prevalence of brucellosis in the Southwestern Rukungiri District are very limited or not available at all. Field investigations and laboratory examinations were carried out to provide further information on the brucellosis situation in this district.

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Using a cross-sectional study design *B. abortus* antibody levels in selected dairy herds were determined by means of the milk ring test (MRT), Rose Bengal plate test (RBPT) and the complement fixation test (CFT). All small ruminants kept on a study farm were also included in the serosurvey. Additional information on management and hygiene risk factors were obtained by means of a questionnaire.

This study, aimed at determining the brucellosis status of dairy herds in Rukungiri District, describes the chosen approach, presents the obtained results and evaluates the applied test systems.

■ MATERIALS AND METHODS

Within a three-month period (September to November 1995) 43 herds of 38 farms consisting of 1094 cattle, 22 sheep, and 102 goats were included in the study (5). The farms had been previously selected by stratified random sampling representing farms with a daily milk yield of at least 10 l delivered to a milking center in the district. The cattle population consisted of the local breed, Ankole, exotic breeds like Friesian, Guernsey and Jersey, and crossbred animals. Cattle were kept on natural but fenced pastures. Approximately 50% of the farmers kept their cattle in

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enclosures overnight, only about 5% of the farmers used communal pastures. Goats and sheep were partly kept together with cattle on pastures, partly held separately in stables and enclosures, or tethered.

Cattle were mainly herded by mixed laborers, whereas family members predominantly took care of small ruminants.

Rukungiri District is in a rural area in Southwestern Uganda with a traditional dairy production system, moving to a more market-oriented system. Milk was delivered to six different milk collecting centers (2). The annual milk yield in 1993 was 3,128,771 liters (unpublished 1993 records from milk collection centers, Rukungiri).

At first the sampling unit was the cattle herd. A bulk milk sample (100-500 ml) was drawn and subjected to MRT. Then MRT prevalence rates were calculated as the number of MRT positive herds divided by the number of MRT tested herds. All herds were covered by one milk sample as none of the herds studied had more than 25 lactating cows.

In a subsequent step blood samples were taken from all cattle and small ruminants older than six months. In herds with more than 30 cattle, 30 animals were selected by simple random sampling. Blood samples were drawn and tested in RBPT. Clear agglutination reactions were regarded as positive. Positive samples were kept at -20°C and later subjected to CFT.

CFT was conducted at the Makerere University of Kampala (Uganda) and in Berlin (Germany) at the BgVV (Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin) according to instructions of the GTZ-BgVV Service Laboratory, Berlin.

B. abortus antigen, *B. abortus* positive and negative controls and complement were produced and provided by the GTZ-BgVV Service Laboratory. Amboceptor was supplied by Behring AG, Marburg, Germany. Alsever solution, hemolytic system (2.5% red blood cells of a local male sheep) and VBD buffer were always freshly prepared prior to use. CFT was performed as cold fixation in microtiter plates. All sera were inactivated at 60°C for 30 min. The last well showing 50% hemolysis represented the titer (dilution up to 1:1280). Sera with anticomplementary activity were regarded as doubtful.

On the basis of CFT results, herd prevalence rates (number of herds with at least one reactor in the CFT/number of herds tested in RBPT) and prevalence rates within the herd (number of CFT reactors/total number of animals per herd or number of CFT reactors/number of serum samples obtained from the herd) were calculated. Individual animal prevalence rates were calculated as the number of CFT reactors divided by the number of serum samples tested.

The agreement between CFT and RBPT was assessed using the kappa statistics (8). Additionally, a questionnaire to collect data on the farming system, milk production and health status of the people living on a farm was administered to the farm or herd manager. In that case the sampling unit represented a herd or farm.

■ RESULTS

Forty-three bulk milk samples were collected on all study farms. As three bulk milk samples could not be used due to spoilage, MRT was performed on 40 samples. Ten percent (4/40) of the herds were found MRT positive. However, only 25.1% (274/1094) of the total cattle population were lactating, 75% of the cattle population were thus not covered by this screening test.

Consequently, in a second step 756 cattle sera from the study were screened in RBPT. Ninety-six (12.7%) were positive and 13 sera (1.7%) showed a slight agglutination and were regarded as doubtful reactors. Given that this RBPT led to a number of doubtful reactions, positive and doubtful sera were retested with a fresh aliquot of RBPT antigen: 30.2% of the sera (33/109) were then found positive, and no questionable or doubtful reaction occurred using that serial test sequence.

CFT was conducted at the Makerere University of Kampala with various modifications. However, none of the sera could be undoubtedly classified due to problems with the hemolytic system. CFT was therefore repeated at the BgVV Service Laboratory (Berlin).

When RBPT and CFT results were interpreted in a serial sequence the following results were reached for cattle sera: 19 out of 33 RBPT positive reactors (57.6%) (normally only RBPT positive sera were subjected to CFT) were confirmed by CFT, four samples showed anticomplementary activity and, thus, were classified as doubtful (12.1%), and 10 sera (30.3%) reacted negatively. Among the 76 sera, which were positive in the first RBPT then negative when retested with a fresh aliquot, four positive (5.3%) and four doubtful (5.3%) CFT reactors were found.

If parallel test interpretation was considered 23 out of 109 samples (21.1%) were then declared positive, 8 (7.3%) samples were anticomplementary and 78 sera were negative (71.5%) in CFT. Based on this interpretation *B. abortus* seroprevalence in individual animals was 3% (23/756) with a confidence interval of 1.8-4.2%. CFT sera reacting anticomplementary were considered negative when calculating the prevalence rates.

The agreement between two tests is quantitatively assessed by calculating the kappa quotient (8). In this study the kappa value calculated for RBPT and CFT was 0.57, respectively 0.60 when sera with anticomplementary activity were also regarded as positive. According to the classification of Thrusfield (8) the agreement between RBPT and applied CFT is considered moderate ($\kappa = 0.4-0.6$).

The highest proportion of seropositive cows was found among the older cows. Fifty-two percent of CFT positive cattle were older than 6 years, 17% between 3-6 years and 30% of the reactors were younger than 3 years. Twenty service bulls, 79 goats and 22 sheep subjected to RBPT were found seronegative. However, one bull of two years of age, not yet introduced as service bull, reacted positively.

The brucellosis prevalence rate of the herds based on the results of the initial MRT was 10% (4/40). One of the MRT positive herds was not confirmed by at least one CFT reactor and was considered MRT false positive. The corrected MRT herd prevalence rate was therefore 7.5% (3/40).

However, if the prevalence rate of the herds was based on CFT results then 25%, 10 out of 40 herds examined, were brucellosis positive herds.

The within herd prevalence rate (table I) showed a wide variability and ranged from 1.5 to 20.4% with a mean of 5.6%. The farm with the highest prevalence rate (36.7% of the animals tested positive in CFT) was a more extensively and traditionally managed farm.

By means of a questionnaire additional information on zoonotic aspects of brucellosis and on husbandry and management factors

Table I

B. abortus within herd prevalence rates in Rukungiri District (September-November 1995)

Herd ID	Herd size	Num. sera tested	MRT result	Num. CFT positive	CFT positive animals /animals tested (in%)	CFT positive animals /herdsize (in%)
1	54	30	positive	11	36.7	20.4
2	31	31	positive	1	3.2	3.2
3	27	27	negative	1	3.7	3.7
4	29	29	negative	1	3.5	3.5
5	47	31	positive	2	6.5	4.3
6	65	37	negative	1	2.7	1.5
7	24	24	negative	1	4.2	4.2
8	72	37	negative	3	8.1	4.2
9	18	18	negative	1	5.7	5.7
10	17	17	negative	1	5.9	5.9
Total	10	384	281		23	mean: 8.0	mean: 5.6

likely to be associated with the herd brucellosis status were obtained. Farmers' knowledge of brucellosis in general was poor. By and large, only half of the farmers could correctly describe the disease. Three farmers reported trustfully that they knew of one or two persons on the farm who had suffered from brucellosis (three cases on positive farms, one case on a negative farm). On most farms aborted fetuses and tissues were left on the pasture or given to dogs. However, significant associations between the herd brucellosis status and factors such as the recent addition of cows to a herd, abortion occurrence, use of breeding bulls and average proportion of pregnant cows were not found.

■ DISCUSSION

Due to complaints from Rukungiri farmers a high brucellosis prevalence rate was expected and MRT was chosen as the initial herd screening test. However, in this study MRT was found unsatisfactory in detecting infected herds. About 75% of CFT positive herds were not detected by MRT. This was mainly attributed to the quite low within herd prevalence rates (5.6%) and to the low proportion of lactating cows within the herds (25%). These results agree with the findings of other investigators (1), i.e., one-time screening of bulk milk samples is insufficient to identify brucellosis affected herds.

RBPT was applied as an initial individual animal screening test to detect infected cattle, sheep and goats. Although RBPT is a well established test, the RBPT test system used first in this study led to a high percentage of false positive and doubtful reactions. All reacting sera in the first test were then subjected to a second test. The correlation of both RBPT was, however, low. The high proportion of false positive sera in the first RBPT was probably the result of a spoiled RBPT antigen aliquot due to unfavorable storage (no constant cooling) under tropical conditions leading to unspecific agglutination reactions. All RBPT reactors of the second test were subsequently submitted to CFT as confirmatory test. However, the normally expected high agreement between CFT and RBPT could not be proven in this study. Residual vaccine titers due to S19 vaccinations could be nearly excluded according to the interviews with farmers, herdsmen, local veterinarians and veterinary assistants. However, the small proportion of false negative reactions in the second RBPT may be

attributed to early stage infections and, theoretically, to ingestion of colostrum of infected dams as well as to vaccination (1). The latter can be excluded in this study as all animals examined were older than 6 months and no vaccination had been carried out. Though a high specificity (98.5%) of RBPT is stated by Stemshorn *et al.* (7) in this study not all sera were correctly classified negative by this test. Overall specificity was increased by serial testing (all reactors of the first RBPT were subjected to a second RBPT). Thus, brucellosis is less likely to be missed, though false positive results are more likely to occur (3).

According to publications and oral reports in Uganda (3, 6) brucellosis prevalence rates were expected to be in the range of 10 to 20%. The assessment of the brucellosis status in this country is often based on empirical observations confirmed by acknowledged tests like MRT, RBPT, ELISA, CFT, as all cases of abortions, stillbirth, but also retained placentas are usually attributed to brucellosis alone.

The overall prevalence rate found in Rukungiri District was 3%, which was substantially lower than prevalence rates previously reported (3, 6). This might be a result of the different test systems used (3: RBPT, MRT and SAT; 6: RBPT and ELISA) and the applied sampling strategy and procedures (survey on extensive and intensive farms, abattoir, etc.). Neither extensively managed/nomadic herds nor beef herds were included in this study as only dairy farms were targeted.

All service bulls, goats and sheep were *B. abortus* seronegative and could be excluded as carriers and disseminators of the disease in the study area. Dogs that had direct contact with aborted materials, e.g., eating afterbirths and fetuses, might have become vectors for *B. abortus*.

On the farm with the highest number of reactors a very poor milking hygiene, sloppy farm management (e.g., no record keeping), poor education and a traditional lifestyle were observed. Unfortunately, no precise data, based on an adequate sample frame and serodiagnosis, could be given on the prevalence rates of bovine brucellosis in humans.

The observation of abortions in seropositive animals seems to be highly associated with a brucellosis infection. Consequently, if abortions were still observed later on these farms, a brucellosis infection should be suspected and investigations and control measures carried out immediately.

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■ CONCLUSION

For a more precise determination of the brucellosis status of the Rukungiri cattle population serological tests, which are reliable under tropical conditions, should be applied. Especially when prevalence rates are expected to be low, the study population has to be screened individually and, preferably, by blood samples only. If bulk milk samples are to be investigated for herd surveillance MRT has to be repeated every 2 to 3 months. This test is of low information value when a cross-sectional study design is selected. RBPT or SAT reactors have to be confirmed by at least one different serological test (for example CFT or ELISA). Considering the different specificity and sensitivity of the tests applied sera should be confirmed in serial and/or parallel test systems. In this study reliable prevalence rates could only be calculated on the basis of individual blood samples tested in parallel with RBPT and CFT.

As on the majority of the infested farms only one reactor within a herd was detected, culling of these animals is the control option to be preferred. On the only heavily infected farm vaccination of all calves with S19 and culling of reactors or vaccination of the whole herd is recommended. However, these measures are probably bound to fail if the farmer is not subsidized by public or private means and has to cover all costs himself.

Due to brucellosis high contagiousness and uncontrolled animal transfer in general it is advisable for all herds in Uganda to be subjected to annual retests. A well established governmental or private veterinary infrastructure and an adequate homogeneous nationwide diagnostic test system need to be set up.

Acknowledgments

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Résumé

Oloffs A., Baumann M.P.O., Afema J., Nakavuma J.
Expériences sur une stratégie de dépistage de la brucellose bovine dans une zone rurale du Sud-Ouest de l'Ouganda

Quarante-trois troupeaux laitiers, comprenant 1 094 bovins, 22 moutons et 102 chèvres, ont été inclus dans une étude sérologique comparative. Le test de réaction d'agglutination (mrt), appliqué comme test de dépistage préliminaire sur des échantillons de lait de mélange, a révélé 10 p. 100 de troupeaux positifs. En raison du faible pourcentage de vaches en lactation dans les troupeaux (25 p. 100 en moyenne), de réactions faussement positives et de la mauvaise conservation du lait, le mrt a été déclaré peu satisfaisant pour détecter les troupeaux positifs. En conséquence, 756 bœufs, 22 moutons et 79 chèvres ont été examinés individuellement avec le test de Rose Bengal (rbpt), puis avec le test de fixation du complément (cft). La correspondance entre rbpt et cft était modérée. En tenant compte des résultats du cft, la prévalence totale de l'infection à *Brucella abortus* était alors de 3 p. 100 (23/756). Cinquante-deux pour-cent des vaches laitières séropositives étaient âgées de plus de 6 ans. Les animaux séropositifs étaient cinq fois plus susceptibles d'avoir eu un avortement observé que les animaux séronégatifs. Le pourcentage de troupeaux infectés était de 25 p. 100 (10/40) et la prévalence de la maladie dans les troupeaux infectés variait entre 1,5 et 20,4 p. 100, avec une moyenne de 5,6 p. 100. Tous les taureaux de monte, les moutons et les chèvres examinés étaient séronégatifs et n'ont apparemment pas joué de rôle dans la transmission de la brucellose dans cette zone. Les tests utilisés (mrt , rbpt et cft) sont discutés et des recommandations sont données pour tenter d'approfondir la recherche sur la brucellose bovine.

Mots-clés : Bovin - Ovin - Caprin - *Brucella abortus* - Brucellose - Réaction d'agglutination - Réaction de fixation du complément - Ouganda.

Resumen

Oloffs A., Baumann M.P.O., Afema J., Nakavuma J.
Experimentos de una estrategia para investigar la brucelosis bovina en una zona rural en el sur oeste de Uganda

Se registraron 43 hatos, incluyendo 1094 bovinos, 22 ovejas y 102 cabras, en un estudio transversal de la brucelosis bovina. El ring test en leche, a partir de muestras de tanques, fue escogido como medio de monitoreo inicial. Diez por ciento de los hatos fueron MRT-positivos. Debido al bajo porcentaje de vacas lactantes en los hatos (en promedio 25 p. 100), a los falsos positivos y al deterioro de las muestras de leche, el MRT no fue satisfactorio para la detección de hatos infectados. Por lo tanto, 756 bovinos, 22 ovejas y 79 cabras fueron monitoreados en forma individual por el test de plato de Rose Bengal (RBPT) y sometidos posteriormente al test de fijación de complemento (CFT). En el presente estudio, el resultado del RBPT y el CFT se encontró moderado. Basados en los resultados del CFT, la tasa de prevalencia de *Brucella abortus* fue de 3 p. 100 (23/756). Cincuenta y dos por ciento de las vacas seropositivas fueron mayores de 6 años. Los animales seropositivos tuvieron cinco veces más posibilidades de haber sufrido un aborto anterior observado que los seronegativos. La tasa de prevalencia del hato fue calculada en 25 p. 100 (10/40) y la tasa de prevalencia dentro del hato varió de 1,5 a 20,4 p. 100 con una media de 5,6 p. 100. Todos los toros de servicio, machios cabríos y corderos fueron seronegativos y no jugaron ningún papel aparente en la transmisión de la brucelosis en el área. Se discute el sistema de tests utilizado (MRT, RBPT y CFT) y se dan recomendaciones para una investigación más profunda de la brucelosis.

Palabras clave: Ganado bovino - Ovino - Caprino - *Brucella abortus* - Brucelosis - Reacción de aglutinación - Prueba de fijación del complemento - Morbosidad - Uganda.

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