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Serosurvey of *Brucella abortus* in cattle and goats in central and southern Uganda

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Abstract

A serological survey was undertaken from April 1990 to February 1993, to determine the exposure experience to *Brucella abortus* in cattle and goats in central and southern Uganda. Three serological tests, the standard tube agglutination test (SAT); rose bengal plate test (RBPT) and the milk ring test (MRT) were used. Samples included blood from slaughter cattle (1,055) and goats (271), both from public abattoirs, as well as live cattle (676) from farms. Milk samples were collected from individual cows (208) on the various farms and from several milk collecting centres (364). For cattle, 14.7% (n=1,731) sera were positive by the RBPT and 12.5% (n=1,731) by SAT. The seropositivity for brucellosis as revealed by testing milk from individual dairy cows was 9.6% (n=208) as opposed to 38.5% (n=364) from milk collecting centres which included milk from local cows. From goats, 8.9% (n=271) were positive by RBPT and 7% (n=271) by SAT. The prevalence was higher on farms with indigenous, 16.15% (n=291), than on farms with exotic breeds of cattle, 7.51% (n=303); and this was attributed to the better sanitary and husbandry methods in farms with exotic breeds. This has been the first comprehensive survey combining detection of antibodies in both serum and milk in cattle in Uganda and the most extensive in the Central and Southern regions of the country in particular. It has revealed a high prevalence of *Brucella abortus* infection among these food animals in the two regions, pointing to both the potential for economic importance as well as the public health risk of this disease. Thus, there is need for instituting control measures.

Key words: Serosurvey, *Brucella arbortus,* cattle, goats

Introduction

Brucellosis is an infectious bacterial disease of sexually mature animals manifested by infection of the joints and the reproductive organs and tracts leading to abortion in females and shedding of the organisms in semen in males (Cruickshank et al.,1968 and Carter, 1979). It leads to economic losses to the farmer as well as being of public health importance (Pelcazar et al., 1978 and Duguid et al., 1978).

The disease occurs worldwide except in certain countries where eradication has been accomplished such as U.S.A (Pelcazar et al., 1978 and Tizard, 1987). An average prevalence of 22.5 ± 1.1% has been shown in some countries of Tropical Africa but may vary from one country to another between 10.4 ± 2% and 40.9 ± 3% (Akakpo et al., 1987). In East Africa, prevalences of 10% in Kenya, 5.8% in Tanzania and 5% in North Eastern Uganda were reported (Kagumba and Nandhokha, 1978). A serological investigation in Rwanda, revealed 35% of the animals positive for brucellosis (Akakpo et al., 1988); while the disease was prevalent in south eastern Zaire in spite of the vaccination programmes (Bula et al., 1987). The first case of human brucellosis in Uganda was investigated in 1910 and the disease occurs in all parts of the country (Aruo, 1973 and Ndyabahinduka, 1978), with several reports of a high incidence of abortions by the field veterinarians. These reports necessitated the determination of extent of exposure of these food animals, especially cattle, to *Brucella* organisms.

The study was carried out in this region because the greatest proportion of the cattle population is located here; the number of exotic cattle breeds in the region is increasing and these are often obtained from countries where the disease has been eradicated and vaccination ceased and are thus very susceptible to infection by *Brucella*.

Materials and methods

Sources and types of animals

The animals, cattle and goats, tested were located or obtained from the Central and Southern regions of Uganda.

Individual farm surveys by use of serum and milk tests. Serum (676) and milk (208) samples were collected and analyzed by the two serum tests and the milk ring test respectively. An interview was used to obtain history about the farms, viz, type of breeding (by insemination or natural service); sources of replacer animals; the vaccination status of the herd and the type of vaccines employed;

occurrence of abortions and the stage of pregnancy at which they occurred and incidence of stillbirths.

Slaughter cattle and goat sera was collected from cattle (1055) and goats (271) at three abattoirs, viz. Kampala meat packers slaughter house and city council abattoir, both in Kampala District; and Kyetume cattle terminal market in Mukono District.

Sample collection

Blood collection. Blood was collected from the severed jugular vessels of the slaughtered animals and from the tail (caudal), or jugular vein in case of the dairy farm cows. Serum was extracted and used for the RBPT and for SAT.

Milk collection. Milk from dairy farms (208 samples) was collected from all quarters of the udder. Bulk tank milk samples (364) were collected once from several milk collecting centres, viz, Kashambo dairy centre for milk collected from Buruli in Luweero District; Nabuka dairy centre; and one collecting centre at Kayunga, both in Mukono District; Kasangati milk collecting centre in Mpigi district; and twelve collecting centres in Mbarara District. The bulk tank milk was thoroughly mixed prior to sampling and about 20 mls were collected from each churn. Milk samples from distant places such as Mbarara District were preserved with Sodium azide and were kept on ice for several days before use in the MRT. All the milk samples were kept at 4°C overnight before use for the milk ring test (MRT).

Serological procedures

Source of antigens. The antigens were purchased from the Central Veterinary Laboratory, Weybridge in England. They included the Rose Bengal *Brucella abortus* antigen, the Standard *B. abortus* agglutination concentrate and the *Brucella* ring test antigen stained with haematoxylin. Before testing commenced, test and control serum and milk samples and appropriate quantities of antigens for the day's work were removed from the refrigerator and allowed to attain room temperature. Positive and negative control sera were included as part of the normal laboratory quality control procedure.

Rose Bengal Plate Test (RBPT)
A drop of serum and then a drop of the Rose Bengal antigen were placed on a ceramic white tile and mixed thoroughly. Agglutination was observed by rocking the tile gently for 4 minutes. The test was carried out following the standard procedure (Morgan et al., 1978).

The Standard Tube Agglutination Test (SAT). The standardized *B. abortus* agglutination concentrate was diluted 1:9 with 0.5% phenol saline to produce the standardized *B. abortus* agglutination suspension. Five serum dilutions were made by the serial double dilution method and an equal volume of the antigen suspension was added. The tubes were incubated at 50°C overnight in a waterbath and read by ordinary light against a dark background. The end titre recorded was the highest dilution giving a positive reaction; and agglutination at a dilution higher than 1/20 was considered SAT positive (Morgan et al., 1978).

The Milk Ring Test (MRT). The standard MRT method as described by Morgan et al., (1978) was used. The milk samples, in 1.0 ml amounts, were placed in narrow test tubes and one drop of the *Brucella* ring antigen added. The contents were mixed by inverting the tubes gently several times, avoiding foaming. The tubes were incubated at 37°C and the results were read after 1 hour.

Results

Slaughter places survey
For the slaughter cattle, 16.3% of the serum samples were positive by RBPT and 13.6% were positive by SAT (n=1,055). Results of the abattoir surveys are summarised in Table 1.

Individual farms survey
The survey results are presented in Tables 2, 3 and 4.

From the screening carried out on various farms, the prevalence of reactors ranged from 0 to 89.5%; with a farm in Masaka District having the highest reactors, 89.5% by RBPT, 84.5% by SAT and 36.8% by MRT (Table 2). There had been no vaccination against brucellosis except on three farms, viz, ITEK Dairy farm, Kyambogo in Kampala District (Table 3), Namaina Dairy farm and Kitetikka farm in

Table 1. Prevalence of brucellosis by the abattoir surveys

Name of abattoir	Animal species	% Positive by RBPT (Total sampled, n)	% Positive by SAT (Total sampled, n)
Kyetume cattle terminal market	Bovine	18.3% (n=437)	15.1% (n=437)
Kampala meat packers slaughter house	Bovine	15.9% (n=251)	14.3% (n=251)
	Caprine	12.4% (n=105)	10.5% (n=105)
Kampala city council abattoir	Bovine	14.2% (n=367)	11.2% (n=367)
	Caprine	6.63% (n=166)	4.82% (n=166)
Total	Bovine	16.3% (n=1055)	13.6% (n=1055)
	Caprine	8.9% (n=271)	7.0% (n=271)

Table 2. Prevalence of *Brucella abortus* on farms with indigenous breeds of cattle (Ankole, Boran and Zebu)

District; name and location of Farm	% MRT positive (Total milk sampled)	%RBPT Positive (Total serum sampled)	% SAT Positive (Total Serum sampled)
Mpigi (A farm at Matugga)	-	0% (30)	0% (30)
Buyana Stock Farm (University Farm)	-	0% (35)	0% (35)
Masaka (A Farm at Sembabule)	36.8% (19)	89.5% (19)	84.2% (19)
Luweero (Various herds)	8.3% (12)	22.2% (90)	21.1% (90)
Mbarara (Various herds)	-	10.3% (117)	8.6% (117)
Total	**25.81% (31)**	**16.84% (291)**	**15.46% (291)**

Table 3: Prevalence of Brucella antibodies on farms with mixed breeds of cattle (Friesian, Ankole, Zebu and their crosses)

District; name and location of farm	% MRT positive (Total milk sampled)	% RBPT positive (Total serum sampled)	% SAT positive (Total serum sampled)
Kampala (ITEK, Kyambogo)	0% (19)	0% (19)	0% (19)
Mpigi (Gayaza J. School)	-	10.5% (19)	10.5% (19)
Masaka (Mukasa's Farm)	-	33.3% (21)	33.3% (21)
Mubende (Namutamba)	-	0% (23)	0% (23)
Total	**0% (19)**	**10.98% (82)**	**10.98% (82)**

Table 4: Prevalence of Brucella antibodies on farms with exotic breeds of cattle (Friesian, Jersey)

District; name and location of farm	% MRT positive (Total milk sampled)	%MRT positive (Total serum sampled)	% SAT positive Total Serum sampled)
Mukono			
Ntawo D.F, I)	-	8.3% (24)	8.3% (24)
Mpoma S.F, Mpoma	-	13.3% (15)	13.3% (15)
Scoul S.F.F, Lugazi	7.1% (14)	12.5% (8)	12.5% (8)
Bulangiti Farm (Mpoma)	-	0% (31)	0% (31)
Others	-	2.08% (48)	2.08% (48)
Mpigi			
Kitetikka Farm	6.5% (31)	-	-
Namulonge Res. Stat.	-	7.5% (40)	10% (40)
Namaina D. Farm	0% (27)	-	-
Jesa Farm, Busunju	0% (8)	0% (8)	0% (8)
Kawanda Res. Station	14.3% (7)	14.7 (34)	8.8% (34)
Liberty D. Centre	-	5.6% (18)	5.6% (18)
A farm at Kiteezi	-	33.3% (6)	33.3% (6)
A farm at Gobero	12.5% (24)	16.6% (24)	12.5% (24)
Kabanyolo Univ. Farm	0% (28)	0% (28)	0% (28)
Heifer Project farmers	0% (4)	0% (4)	0% (4)
Jinja			
Bugembe Isl. School	20% (15)	20% (15)	20% (15)
Total	**6.96% (158)**	**7.92% (303)**	**6.6% (303)**

Mpigi District (Table 4). These farms employed the dead vaccine S45/20 and only once, no booster vaccinations had been given.

The average prevalence of brucellosis in the various farms in districts was as follows: Luweero District had the highest prevalence (22.2% by RBPT; 21.1% by SAT and 8.3% by MRT), followed by Mbarara (10.3% by RBPT and 8.6% by SAT), Mpigi (6.9% by RBPT; 6.1% by SAT and 6.2% by MRT) and Mukono Districts (4.8% by RBPT; 3.2% by SAT and 7.1% by MRT) in this order. Each of Kampala, Jinja and Mubende Districts had a herd from only one farm screened and the results revealed a prevalence of 0%, 20%

and 0% respectively. Masaka District had a prevalence of 60% by RBPT; 57.5% by SAT and 36.8% by MRT, but only two farms were screened from the district (Tables 2 &3).

Serum samples collected and analysed from the individual farms survey were 676, of which 82 (12.1%) were positive to RBPT and 74 (11.0%) were positive to SAT. A total of 208 milk samples were collected from the various farms, of which 20 (9.6%) reacted positively to MRT.

Bulk milk screening from various collecting centres

Some milk collecting centres from 4 districts had their bulk milk screened by MRT. Each bulk sample represented from 3 to 50 animals. Milk collected at Kayunga collecting centre showed the highest reactors (57.14%); followed by that from Kashambo at Kawempe, originating from Buruli in Luweero District (55.6%); milk from the collecting centres in Mbarara District (43.7%); milk from Nabuka dairy centre (8.82%); and milk from Kasangati collecting centre (8.2%). By districts, Luweero had the highest reactors (55.6%), followed by Mbarara (43.7%), then Mukono (27.3%) and lastly, Mpigi District (8.16%) [Table 5].

Discussion

Seroprevalence of *Brucella abortus* in goats

Antibodies to *Brucella abortus* were detected in abattoir surveys in goat sera (Table 1). However, these were based on comparatively fewer samples than for cattle. Goats had a prevalence of 7.95%. *Brucella abortus* has cattle as the primary host, although, occasionally it infects sheep and goats when these animals are kept in close contact to cattle. Thus, the positive reactions in goats were probably due to the presence of antibodies produced in response to either *B. abortus* or *B. melitensis* infections. Since these two species share the A and M epitopes, cross reactions may have occurred when the goats were actually infected with *B. melitensis* (Carter, 1979). The positive reaction could also have been due to infection with other non-Brucellae which cross-react with *B. abortus* antigen (Nielsen and Duncan, 1990). Isolation and typing of Brucellae would have confirmed the cause of positive reaction to the tests.

Prevalence of *Brucella abortus* reactors in cattle in central and southern Uganda

Brucellosis can seriously affect dairy cows in Uganda resulting in economic losses. Previous serological surveys in Uganda have indicated high prevalence of the disease in cattle (Aruo, 1973).

By this study, results of the brucellosis serological survey in abattoirs indicated a high prevalence of *Brucella abortus* infection in cattle, being 14.95% [average by the two tests, since their results correlated highly] (Table 1). This is considered high because it exceeds the 4% prevalence in the animal population, which level is necessary for eradication control strategy to be initiated (Blood et al., 1983). The average prevalence levels of brucellosis in cattle from the abattoir and dairy farm surveys was 13.6%. The prevalence levels differ from those reported by Kagumba and Nandokha, (1978) of 5% in N.E Uganda probably due to environmental differences, where the N.E Uganda region is drier and less humid than the Central and Southern regions, thus, the organisms do not survive for long in the environment. An early report of 18% on one farm (Aruo, 1973), emphasizes the observation that the prevalence is still high since the farm was located in the present region of study.

The high prevalence of bovine brucellosis in Uganda in this study and others (Aruo, 1973 and Kagumba and Nandokha, 1978) can be generally attributed to a number of factors.

Firstly, the management practices, especially communal grazing and transhumance involving large herd sizes, results in a low value being attached to abortions as many calves are still raised. Secondly, during disease control programmes, priority is given to diseases with high morbidity and mortality such as rinderpest, East Coast Fever (ECF) and Contagious Bovine Pleuropneumonia (CBPP); thus neglecting brucellosis control. Thirdly, lack of ample financial resources has resulted in use of available funds to control diseases that are considered to be most important, or more widespread viz, ECF, trypanosomiasis, rabies and FMD; hence control of brucellosis through vaccination has received little attention, although vaccines are available. Finally, vaccination against brucellosis is voluntary and therefore not subsidized by government.

Prevalence of brucellosis in indigenous and exotic cattle

In Uganda, most cattle are of indigenous breed, with a population of 3,714,305 animals in comparison to 109,999 exotic cattle (MAAIF, 1989). Therefore, the majority of the cattle that were screened, especially during the abattoir surveys were of the indigenous type. Of the exotic cattle, which are kept for dairy purposes, only those culled because of infertility, or unthriftiness, or due to terminal illness were taken for slaughter. The abattoir surveys revealed high prevalences of 16.3% by RBPT and 13.6% by SAT.

Table 5. Prevalence of Brucella antibodies as revealed by the Milk Ring Test on bulk milk samples

Collecting centre and location	District	Cattle breed	% MRT positive (Total sampled, n)
Kasangati milk coll. centre (Kasangati)	Mpigi	Exotic	8.16% (n=49)
Kashambo coll. centre (Kawempe)	Luweero	Local	55.6% (n=63)
Various centres (Mbarara)	Mbarara	Local, Crosses	43.7% (n=197)
Nabuka dairy centre	Mukono	Exotic, Crosses	8.82% (n=34)
Kayunga coll. centre	Mukono	Local	57.14 (n=21)
Total			**38.5% (n=364)**

The individual farm surveys revealed that herds with exotic breeds of cattle had comparatively fewer reactors to *B. abortus* antigens by the serum tests (23 out of 322, Table 4), as opposed to farms with indigenous breeds, which had more reactors (Tables 2, 3 and 4). Districts that had farms with indigenous cattle screened showed higher prevalences, for instance, Masaka, Luweero and Mbarara Districts in comparison to those districts where the majority of the herds that were screened had exotic cattle, such as, Mpigi and Mukono Districts. For the latter, the majority of the herds that were screened consisted of exotic cattle and these showed lower seroprevalences than where indigenous cattle had been tested. This is further indicated by the bulk milk surveys, where collecting centres with contributing farms consisting of exotic cattle showed the least reactors, Nabuka dairy centre and Kasangati milk collecting centre. Kayunga collecting centre and Nabuka dairy centre are both located in Mukono District, but the former received milk from indigenous cattle and the latter had milk from the exotic cattle and thus, the different percentage of reactors. Collecting centres where the contributing farms had indigenous cattle had the highest reactors, such as, Kashambo in Luwero (55.6%), Kayunga in Mukono (57.14%) and Mbarara (43.7%).

The differences in prevalence of bovine brucellosis in the indigenous and the exotic breeds in Uganda may be related to a number of factors. The management practices especially communal grazing and transhumance, commonly practiced by farmers with indigenous breeds play a role in disseminating the disease over a large area. This relates to the high prevalence reported in neighbouring countries where transhumance is also practiced, such as 10% in Masaailand of Kenya (Kagumba and Nandokha, 1978) and 35% in Rwanda (Akakpo et al., 1988). For the exotic cattle, husbandry is of a modern type involving either zero grazing for farmers with few animals, or rotational grazing on farms with medium to large size herds having well fenced paddocks. There is high hygienic standards, the calving pens are cleaned and disinfected, afterbirths are disposed of by burrying or burning. With rotational grazing, the resting period of a paddock is enough to allow any pasture contamination to die out before the animals are returned to the same paddock. With communal grazing, there are no precautions taken to decontaminate soiled pastures.

Transhumance has contributed highly to the spread of diseases to various districts, as indicated by the high prevalence of brucellosis in cattle in Masaka and Luweero Districts. The majority of farmers keep indigenous animals in large numbers without considering the degree of productivity but rather for prestige and as insurance against financial and drought hardships. Most of them lack the knowledge on disease prophylaxis, thus, vaccination against brucellosis is rarely carried out even on farms with exotic animals.

The few reactors that were encountered on farms with exotic breeds of cattle were due to the fact that most of these farms obtained replacer animals from other farms as identified from the oral interviews. The status of the animals as far as brucellosis is concerned was never established before the animals were introduced to the farms. However, most of the farms with exotic cattle practiced artificial breeding using quality semen obtained from Entebbe Artificial Insemination Centre.

The prevalence of bovine brucellosis was high, thus there is need to apply control measures, such as, informing the farmers on the various control measures both specific and non specific.

They should practice good farm management like establishing the brucellosis status of the replacer animals especially where they are obtained from elsewhere. Calfhood vaccination should be advocated followed by regular testing to monitor the levels of brucellosis. Where transhumance is on a great scale, mass vaccination should be carried out to enhance herd immunity. The animals should be tested and isolated into reactor and non-reactor herds; and cattle in the suspect herds should be serologically examined and presence of infection confirmed by isolating the Brucellae. All the above measures should be coupled with hygienic procedures to prevent spread of the disease in a herd or to other herds.

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