

Comparative *Brucella abortus* antibody prevalence in cattle under contrasting husbandry practices in Uganda

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A study was conducted in the Luwero and Nakasongola districts in central Uganda to determine and compare the prevalence and distribution of antibodies against *Brucella abortus* in cattle under contrasting husbandry practices, using two serological tests. Three hundred and fifteen serum samples were systematically sampled from 29 farms and subsequently tested using the Rose Bengal plate test (RBPT) and Indirect Antibody Enzyme Linked Immunosorbent Assay (I-ELISA). The overall prevalence of antibodies against *Brucella abortus* in the Nakasongola and Luwero districts was 2.4% and 4.7% on RBPT, compared with 1.2% and 3.34 % on I-ELISA. There was no significant difference between the results obtained by RBPT and indirect antibody ELISA ($p > 0.05$). It was noted that antibodies against *Brucella abortus* were widely spread over different farms regardless of the cattle grazing system ($p > 0.05$). Based on the findings, it is feasible to use RBPT as a cheaper screening alternative for brucellosis. A comprehensive national brucellosis study should be undertaken to study the epidemiology and prevalence of brucellosis in Uganda.

Introduction

Bovine brucellosis is an infectious and contagious bacterial disease that largely affects mature domestic and wild animals (Mwebe, Nakavuma & Moriyón 2010). It is characterised by inflammatory changes in the foetal membranes that lead to premature expulsion of the foetus (Kungu *et al.* 2010). Brucellosis is a major public health hazard that is persistent in many communities in the world and is one of the neglected endemic zoonoses that requires substantive attention (FAO 2009; Kabagambe *et al.* 2001). It is caused by coccobacilli of the genus *Brucella*, species *Brucella abortus*, which is a facultative intracellular parasite.

Brucellosis causes economic losses through decreased animal productivity, abortions and infertility in up to 20% of cattle (FAO 2009; Mwiine 2004). This contributes to persistence of poverty amongst farmers in developing countries, as the disease is also considered to be a major impediment to export of animal products (Mangen *et al.* 2002).

Brucellosis in humans presents with febrile 'flu-like illness, frequent chills, headaches and general weakness (Krause & Hendrick 2010). Humans get infected through consumption of raw milk, via skin abrasions or mucous membranes and inhalation. In cattle it is usually spread by the vaginal discharge of an infected cow or an aborted foetus. Infected breeding bulls can transmit the disease to cows at the time of service by infected semen.

The threat of bovine brucellosis in Uganda is expected to increase much more since livestock is steadily increasing, as evidenced by the livestock census of 2008 that showed an increment from 8.4 million in 2006 to 11.4 million in 2009 (UBOS/MAAIF 2009).

Several factors, such as livestock production systems, herd size, limited vaccine coverage and availability, interaction with wildlife, ecological and socio-economic factors, are important in the epidemiology of the bovine brucellosis (Kabagambe *et al.* 2001). Grazing systems have been reported as important factors in the epidemiology of bovine brucellosis (Kungu *et al.* 2010). Cattle grazing systems in Uganda differ widely, with the majority being communal (non-paddocked farms).

As brucellosis is a disease with public health significance and there is a lack of information existing in Uganda, this study was aimed at generating baseline information that could help in planning control programmes. A previous retrospective study done by Mwebe, Nakavuma & Moriyón (2010) indicated the presence of brucellosis in the Luwero and Nakasongola districts in Central Uganda, but there is little information about the prevalence and distribution of bovine brucellosis under the contrasting grazing systems in Uganda.

Brucellosis diagnosis is still challenging because of a number of false positives with most screening tests, but confirmation can be achieved by isolation using plain or selective media by culture of *Brucella* organisms. However, this presents a number of drawbacks like slow growth,

low sensitivity due to differences in *Brucella* species, culture medium requirements, and the number of bacteria that will be detected by the culture technique employed (Abubaker *et al.* 2010). Due to the high biosecurity standards required, most laboratories in Uganda do not undertake culturing of *Brucella* organisms. There is therefore a need to research the use of readily available serological diagnostic techniques including Indirect Antibody Enzyme Linked Immunosorbent Assay (I-ELISA) and the Rose Bengal Plate Test (RBPT).

The main objective of this study was to determine the prevalence and distribution of the antibodies against *Brucella abortus* in cattle under contrasting husbandry practices. The data would enable evaluation of the current situation and provide information about the control programmes undertaken by the different stakeholders.

Materials and methods

Study population and sampling

The study, to determine the prevalence of antibodies against bovine brucellosis was done in collaboration with the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) during routine disease surveillance in October 2010. The Luwero and Nakasongola districts in central Uganda were selected based on the differences in cattle grazing systems and previous reports about brucellosis. A study population of cattle was selected and blood samples were collected for laboratory analysis. The sample size of each district was calculated at 5% level of precision, 95% confidence level using the formula adapted from Thrusfield (1995) as follows: $n = Z^2P(1-P)/D^2$. The required sample size is n ; Z is the multiplier from normal distribution equivalent to 1.96, P was the estimated crude prevalence, $(1-P)$ was the probability of having no disease and D was the desired precision (5%), confidence interval 95%. Based on previous studies in Uganda by Mwebe, Nakavuma & Moriyón (2010) and Nakavuma (1994), crude prevalence for Luwero was estimated at 14% and Nakasongola was estimated at 12%, therefore a calculated sample size of 185 and 168 serum samples was targeted from 29 selected farms.

Of the 29 farms, 14 farms were from Luwero district (eight non-paddocked and six paddocked) and 15 farms were from Nakasongola (eight non-paddocked and seven paddocked) see Figure 1. The sampling unit was a sub-county where 20 serum samples were collected. This was determined by dividing the sample size per district by the number of sub-counties or sampling units. Out of 353 targeted sera samples, only 315 were collected based on farmer compliance and turnout.

Blood was collected from either the jugular or coccygeal vein using sterile plain vacutainer tubes. Sampling information taken included the farm number based on where sampling began, date of sampling, month and year, grazing system, herd size, GPS coordinates, sub-county, parish, village and district. Samples were put in cool box with ice and transported to the NADDEC laboratory in Entebbe where the sera were

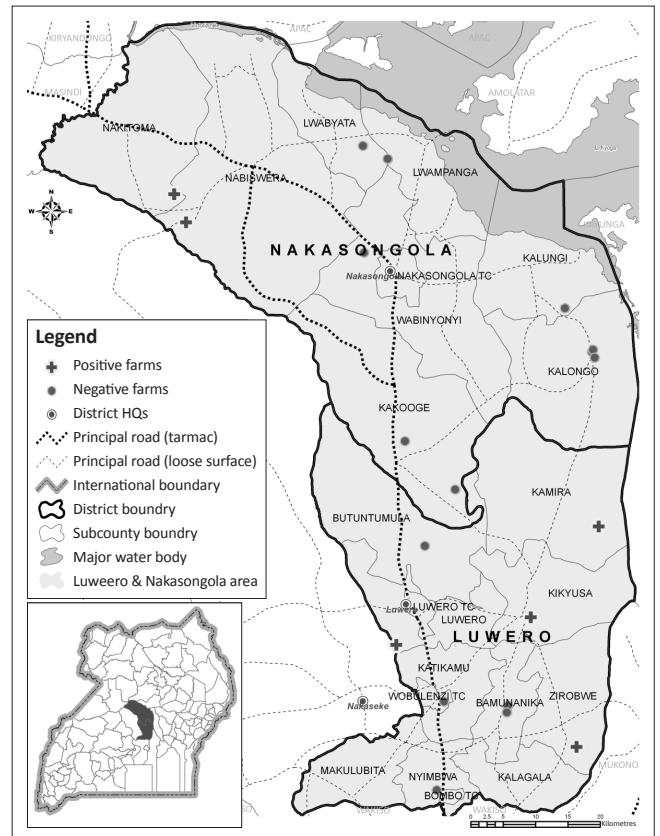


FIGURE 1: Map of Uganda showing two study districts of Luwero and Nakasongola.

aliquoted into small serum vials and stored in freezers at -20 °C until the time for analysis.

Serology

Anti-*B. abortus* antibodies were detected by serial testing of sera using the Rose Bengal Plate Test (RBPT) (Alton *et al.* 1975) and Indirect Antibody Enzyme Linked Immunosorbent Assay (I-ELISA) (SVANOVIR® Biotech AB, Uppsala, Sweden). A true positive sample was one that was positive to both RBPT and I-ELISA.

All serum samples were exposed to *B. abortus* smooth lipopolysaccharide-coated wells by adding 100 µl of sample dilution buffer followed by 4 µl of serum samples and incubated for one hour at 37 °C. Anti-mouse IgG antibody conjugate horseradish peroxidase was added to all wells and again incubated for one hour, and plates were washed and rinsed to remove unbound antigen. A substrate solution was added to all wells and incubated at room temperature for 10 min. A stop solution (H₂SO₄) was added before the results could be read. A blue colour indicated a positive reaction due to conversion of conjugate by the substrate (I-ELISA bench protocol). The optical density (OD) for the serum and controls was read at 450 nm in a micro-plate photometer linked to a computer. The percentage positivity (PP) was determined using the following formula: PP = OD Sample or Negative Control x 100/OD Positive Control.

The test samples were considered to be positive if the OD value obtained was equivalent to or larger than the PP or inhibition of 40%. The cut off percentage positivity was 40.

Data analysis

Sampling data and laboratory results were validated and entered into a Microsoft Excel spreadsheet. A map to show the distribution of antibodies was drawn based on the geographical coordinates of sampled farms using Arc View GIS. Statistical comparisons, to compare results obtained by RBPT and I-ELISA and the prevalence of antibodies between paddocked and non-paddocked farms, were done using a t-test statistic and graph pad prism version 5.0 at $p < 0.05$, confidence interval of 95%.

Results

The total number of serum samples tested by both RBPT and I-ELISA was 315. Out of 149 samples from Luwero, seven samples (4.7%) tested positive for antibodies against *B. abortus* by RBPT, whilst four samples (2.4%) out of 166 from the Nakasongola district tested positive. Using I-ELISA, five out of 149 samples (3.34%) from the Luwero district tested positive, whilst only two out of 166 samples (1.2%) tested positive from the Nakasongola district (Table 1).

Luwero Town Council and Kamira sub-counties recorded a prevalence of 10% ($n = 2$), followed by Kikyusa 9.1% ($n = 1$), Ziobwe 5% ($n = 1$) and Nyimbwa, 5% ($n = 1$) by RBPT. Serum samples from Butuntumula, Bamunanika and Katikamu subcounties tested negative to both RBPT and I-ELISA. Kamira sub-county showed the highest prevalence by I-ELISA (10%), followed by Kikyusa (9.1%), then Luwero and Ziobwe with a prevalence of 5% (Figure 2).

Nabiswera sub county recorded the highest prevalence of antibodies against *B. abortus* of 12.5% ($n = 2$), followed by Kalungi and Nakitoma at 5% ($n = 1$) using RBPT. Nabiswera sub-county had the highest prevalence of 6.25%, followed by Nakitoma (5%) by I-ELISA. Serum samples from Kakoge, Kalongo, Lwabyata, Lwampanga, Wabinyonyi, Nakasongola town council tested negative to both RBPT and I-ELISA (Figure 3).

Grazing systems

Out of 29 farms sampled from the Luwero and Nakasongola districts, seven had at least one animal testing positive for antibodies against brucellosis. Out of the seven positive farms, five were non-paddocked, whilst two were paddocked. Luwero district had more positive non-paddocked farms ($n = 4$) than Nakasongola ($n = 1$), but they had the same number of positive farms that were paddocked (Table 1).

A farm was considered positive if at least one animal tested positive to both RBPT and I-ELISA upon serial testing.

Discussion

The overall prevalence of antibodies against *B. abortus* in the Nakasongola and Luwero districts was 2.4% and 4.7% on RBPT, compared with 1.2% and 3.34% on I-ELISA, respectively. This was lower than the prevalence reported by Mwebe, Nakavuma & Moriyón (2010), who found 11% and 17% for the Luwero and Nakasongola districts respectively. This was possibly due to the differences in sampling since the retrospective studies were based solely on clinical cases and submissions from field veterinarians.

This study also contrasts with other surveys in various parts of Uganda by researchers who reported varying prevalence: Nakavuma (1994) reported 14.7% in the Central Region,

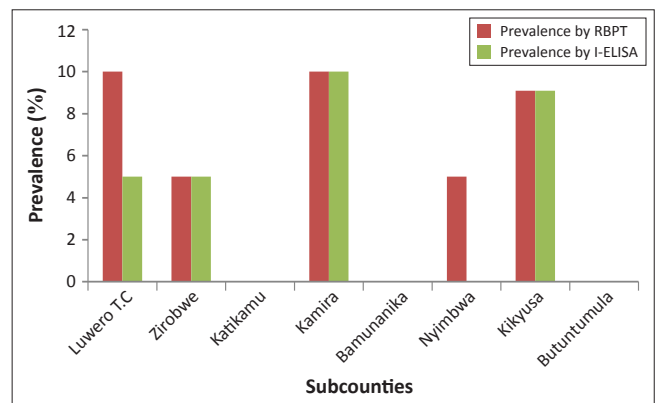


FIGURE 2: Prevalence of antibodies against *Brucella abortus* by sub-counties in the Luwero district.

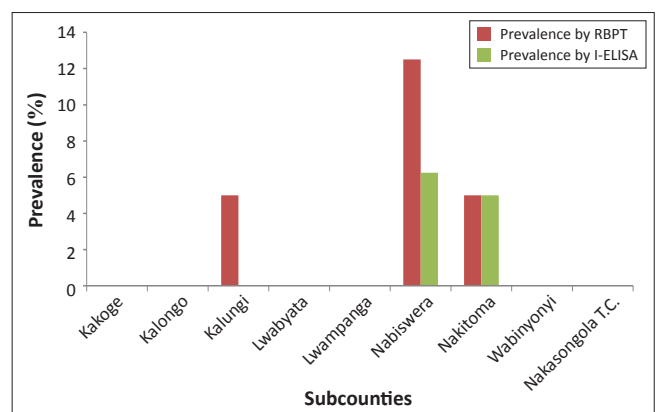


FIGURE 3: Prevalence of antibodies against *Brucella abortus* by sub-counties in the Nakasongola district.

TABLE 1: Grazing systems of sampled farms in the Luwero and Nakasongola districts, Uganda.

District sampled	Paddocked				Non-paddocked			
	Farms sampled	Positive farms	Positive samples	<i>n</i>	Farms sampled	Positive farms	Positive samples	<i>n</i>
Luwero	6	1	1	69	8	4	4	80
Nakasongola	7	1	1	73	8	1	1	93
Total	13	2	2	142	16	5	5	173

Prevalence of *Brucella abortus* antibodies was 2.8% and 6% from paddocked and non-paddocked farms respectively.

A farm was considered positive if at least one animal tested positive to both RBPT and indirect antibody ELISA upon serial testing. It was also shown that grazing difference did not have a significant contribution to the prevalence of antibodies against *Brucella abortus*.

n, given as a number.

$P > 0.05$; $P = 0.227$.

Kagumba & Nandoka (1978) reported 5% in North Eastern Uganda and Ndyabahinduka (1978) reported 18.1% in East Ankole and the Central Region. The differences in reported prevalence was due to temporal, spatial, sampling and assay differences, and they highlight the fact that there is a potential problem of brucellosis in many parts of Uganda. It is not possible to rule out the possibility that the incidence of brucellosis may vary from time to time due to control interventions and good husbandry practices.

Sub-county level prevalence of antibodies against *B. abortus* tested by RBPT indicated that 62.5% ($n = 5$) of the sub-counties in the Luwero district had antibodies circulating in their herds compared to 50% ($n = 4$) tested by I-ELISA of the sub-counties in the Nakasongola district. The presence of antibodies indicated by both RBPT and I-ELISA was 33.3% ($n = 3$) in the sub-counties of the Luwero district and 22.2% ($n = 2$) in the sub-counties of Nakasongola.

In this study there was no significant association ($p > 0.05$, $p = 0.227$) between the two categories of grazing systems (paddocked and non-paddocked grazing systems) and the prevalence of antibodies against *B. abortus*. In contrast, Kungu *et al.* (2010) found that non-paddocked farms in the Gulu and Amuru districts were at high risk of having brucellosis compared with paddocked farms (OR = 4.26). In this study, antibodies were distributed throughout the two districts with no clear pattern of relationship with the system of grazing. This is perhaps due to the low prevalence of brucellosis detected in the study area.

The presence of antibodies against *B. abortus* in cattle sera samples from the districts studied concurs with previous studies by other researchers that brucellosis occurs throughout Uganda (Kabagambe *et al.* 2001; Mwebe, Nakavuma & Moriyón 2010; Nakavuma *et al.* 1999). The prevalence of antibodies against *B. abortus* in these districts was most likely due to incidental infections since there was no history of vaccination in the sub-counties studied.

The RBPT showed a higher prevalence of antibodies over I-ELISA for serum samples from the districts studied. This was probably due to differences in specificity and sensitivity between the tests, as reported by Poester, Ramos & Thiesen (2010) who found the specificity and sensitivity of RBPT to be 100% in natural infections compared with I-ELISA at 98.2% and 98.6% respectively. In this study, although the tests were not statistically any different ($p > 0.05$) in performance in the Luwero and Nakasongola districts, RBPT would be the preferred test. This is because the cost, ease of use and performance of RBPT offer comparative advantages over I-ELISA during screening of large herds of cattle. These findings are in conformity with other studies (Dohoo *et al.* 1986; Sutherland 1984) where RBPT was found to be a good screening test, although others, such as Saravi *et al.* (1992), found an unacceptable false negative rate with RBPT.

High sensitivity of RBPT was due to its ability to detect more positives than I-ELISA. This is in agreement with findings of Poester, Ramos & Thiesen (2010), who found the sensitivity of RBPT to be 100% compared with I-ELISA,

which was 98.6% from *Brucella* infected animals. However, the disadvantages of RBPT are that antibodies from other bacterial infections like *Yersinia enterocolitica* and *Salmonella* can cross react with smooth lipopolysaccharides, thereby confounding the interpretation of RBPT results, and it also does not distinguish between vaccinated and infected animals (Radostits *et al.* 2000).

The authors therefore conclude that the prevalence of antibodies against *B. abortus* was not as high in the two districts as previously reported. There was also no significant difference between results obtained by RBPT and I-ELISA tests and therefore RBPT could offer an alternative for screening herds in developing countries. Grazing systems did not contribute significantly to the prevalence of antibodies against *B. abortus*.

In spite of the low prevalence of antibodies in the study areas, the fact that brucellosis is a potential public health hazard and that positive samples were detected on many different farms shows that the risk of exposure to infection for animals and human beings is still high. Vaccination of cattle is pivotal in the control of this disease. A national survey to establish the prevalence of *B. abortus* and, more importantly, to establish the biotypes should be undertaken. Such a study could provide the information needed for mass testing, slaughter and awareness campaigns to eradicate brucellosis in Uganda.

Conclusion

In conclusion, the authors observed that the prevalence of antibodies against *B. abortus* was not as high in the two districts as previously reported. There was also no significant difference between the results obtained by RBPT and I-ELISA tests and as such RBPT could offer an alternative for screening herds in developing countries. Grazing systems did not contribute significantly to the prevalence of antibodies against *B. abortus*. A national brucellosis study to establish the prevalence of *B. abortus* should be undertaken and more so to establish the biotypes.

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Competing interests

There are no competing interests that any of the authors are aware of.

Authors' contributions

The research was done as part of a Bachelors degree special project at the National Animal Disease Diagnostics and Epidemiology Centre (NADDEC) referral laboratory. G.N. (NADDEC) did the practical work and was supervised by C.A. (NADDEC) and F.N.M. (Makerere University); we wrote and submitted the manuscript together.

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