The prevalence of bovine brucellosis in milking dairy herds in Nyagatare and its implications on dairy productivity and public health

Chatikobo P*, Manzi M2, Kagarama J1, Rwemarika JD2, Umunezero O2
1Umutara Polytechnic, Faculty of Veterinary Medicine, P.B 57, Nyagatare, Eastern Province, Rwanda; E-mail: paulkobo@gmail.com; paulchatie@yahoo.com; 2Institute des Sciences Agronomiques du Rwanda (ISAR), Nyagatare Livestock Production & Health Research Unit, B.P 5016 Kigali, Rwanda; *Corresponding author

Key words: bovine brucellosis, zoonotic disease, infertility, dairy herds, Rwanda

Abstract

Between April and June 2008, 998 serum samples from 205 herds located in 10 different sectors within the district were screened for brucellosis using Rose Bengal Plate test. Out of a total of 998 serum samples tested, 99 (9.9 %) reacted positive for brucellosis using the Rose Bengal Plate Test (RBPT). Bovine brucellosis was detected in nine out of the ten sectors in Nyagatare, and out of the 205 herds studied, 62 were seropositive. The overall brucellosis herd prevalence rate (HP), i.e. at least one positive RBT reactor identified in a herd, was associated with sector ($X^2 = 8851.228, P = 0.000$), Breed ($X^2 = 413.567, P = 0.002$), and parity of the cow ($X^2 = 580.292, P = 0.000$). Significantly higher brucellosis herd prevalence values were reported for Byera (100 %), Katabagemu (45.45 %), and Rwimbogo (42.86 %) sectors. The herd prevalence was 29.62 % in Ankole cattle (95 % CI: 28.36 to 30.87) and 23.71 (95 % CI: 17.23 to 30.19) in purebred Friesian-Holstein cattle, with a statistically significant difference ($X^2 = 413.567, P = 0.000$). Individual animal prevalence (IAP), i.e. number of individual positive reactors, differed ($P < 0.05$) between and within the sectors, and was also associated with the breed of the cow. Significant higher overall IAP’s were found in Byera (20 %), Rwimiyaga (12.17%), and Rwimbogo (12.00 %). Individual animal prevalence was 9.75 % (95 % CI: 9.34 to 10.16) in Ankole cattle and 7.15 % (95% CI: 5.46 to 8.84) in Purebred Friesian-Holstein cattle with a statistically significant difference ($X^2 = 335.339, P = 0.000$). There was no statistically significant difference in individual prevalence between Ankole cows and crossbred cows. On the other hand, the prevalence of brucellosis in cattle was also found to be higher in the older parities than younger ones. Overall seropositive reactors recorded were 12/204 (5.9 %) for parity 1, 20/181 (11.05 %) for parity 2, and 11/77 (14.29 %) for the fourth parity cows. However, no statistically significant difference was observed in the prevalence of brucellosis between male and female animals. Overall, the study reveals that bovine brucellosis is endemic in Nyagatare. The public health and livestock productivity implications of the present findings are discussed.

Background and justification

Bovine brucellosis is a highly contagious systemic bacterial disease caused by Brucella abortus (12, 7). It is primarily a disease of ruminants (5), and is regarded as one of the most widespread zoonoses in the world (13). The disease is of economic importance in dairy production because it adversely affects the reproductive and productive potential of dairy cows and is a major impediment for trade and export of livestock products (28). Infection in pregnant cows is characterized by abortion, birth of dead or weak calves, retained placenta, endometritis, repeat breeding, infertility, as well as reduction or complete loss of milk yield after the abortion. In bulls the disease results in testicular lesions such as orchitis, epididymitis, and seminal vesiculitis which affect their breeding capacity.

Besides the impacts of the disease on livestock, brucellosis is also an important zoonoses,
more commonly known as undulant fever. Infection in human beings result in chronic debilitating illness which requires prolonged treatment. The established mode of transmission of *Brucella* spp. to humans is usually by direct contact with infected animals or their carcasses (32), or through ingestion of infected unpasteurized milk or dairy products (12). Affected humans develop a chronic debilitating, on- and off (undulating) febrile flu-like illness (8, 33), that is frequently confused with malaria or typhoid with the result that inappropriate treatment is often given. The course of the disease is prolonged leading to considerable medical expenses in edition to loss of income due to loss of working hours.

Brucellosis is of particular public health importance in societies that live closely together with their livestock. In Rwanda, about 92% of the population live in rural areas and depends on agriculture for survival. The rural areas or ‘villages’ in Nyagatare are generally regarded as resource-poor areas with a weak infrastructure, a high unemployment rate and subsistence livestock farming dominate over other agricultural activities. As observed elsewhere (21), consumption of unpasteurised milk, undercooked or fresh meat are not uncommon in the rural households. However, very little is known about the prevalence of important zoonotic and production diseases of cattle in these areas, which is essential information for the prioritization and implementation of disease control schemes.

In our previous study (6), we observed unusually higher incidences of abortion, retained placenta, and infertility of unknown origin in dairy cattle in Nyagatare, Gatsibo, and Kayonza Districts. Although these symptoms are commonly seen in brucellosis infected herds (29, 22, 21), there is no documentation about the occurrence of this disease in this district. To gain an understanding of the prevalence of brucellosis in Nyagatare, and to seek possible explanations on the causes of abortion in dairy herds and devise appropriate control strategies in the area a large scale serological brucellosis screening survey was undertaken.

**Materials and methods**

**Study site**

Nyagatare is located in Eastern Rwanda, bordering Uganda, and Tanzania. The district is about 150 km away from Kigali, the capital city. Nyagatare, Gatsibo, and Kayonza (the former Umutara Province) hold about 40% of the cattle population of the country currently estimated at just over 1.2 million. While some of the milk produced in the district is sold at big urban markets in Kigali, most of it is sold through the informal market within the district. Presently the province is in a transition phase from the extensive traditional husbandry to the market orientated systems. The cattle population consists of predominantly local Ankole types and various crosses between these and exotic breeds, raised in extensive traditional husbandry system. However, there is an increasing proportion of introduced purebred cattle such as Friesian- Holsteins, Jerseys and Guernsey. Both cattle and small ruminants are often grazed or tethered together. All study herds were selected by stratified random sampling, milk collection centers being the strata. The criteria for selection of herds was the supply of milk to local milk collection centre, and high reported prevalence’s of abortion, and retained placenta of unknown origin in previous studies (6).

**Blood Sample Collection**

About 10 mL of blood was collected form the jugular or coccygeal vein of each selected animal using plain vacutainer tubes and allowed to clot overnight at room temperature. The serum samples were separated and transported in iceboxes to Rwanda Animal Resources Development (RARDA) Veterinary Research and Diagnostic
Laboratory, in Kigali and stored at -20°C until testing.

**Serological detection of Brucella antibodies**

At RARDA, the Rose Bengal Plate test (RBPT) was used to screen the serum samples to detect the presence of *Brucella* agglutinins. Serum samples from cattle were tested using RBPT according to standard methods as described by (1, 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 minutes. Presence of agglutination was regarded as positive.

**Data analysis**

The data collected in the field were entered into a computer on a Microsoft Excel spreadsheet. Statistical analysis (multivariate logistic regression) was performed using ‘Statistical package for the social sciences’ (SPSS), version 11.5 (for Windows). The prevalence proportion was calculated as the number of animals testing positive by the RBPT, divided by the total number of animals tested. Three epidemiological parameters were generated, the herd prevalence, within-herd prevalence, and individual prevalence. Herd prevalence was calculated by dividing the number of herds with at least one reactor in RBPT by the number of all herds tested (Equation 1). The within-herd prevalence was calculated by dividing the number of RBPT reactors within a herd by the number of serum samples tested in the herd (Equation 2). The individual or total prevalence was calculated by dividing the number of RBPT positive animals by the total number of animals tested (Equation 3). Equations below show how the three epidemiological parameters were derived.

1. **Herd prevalence** = number of herds with at least one positive reactor
   Number of herds sampled

2. **Within-herd prevalence** = number of positive reactors
   Number of serum samples tested from this herd

3. **Individual animal prevalence** = number of individual positive reactors
   Number of serum samples tested.

Analyses were carried out to compute proportions of seropositive animals (stratified by sector, breed, sex, and parity where relevant) and their 95% confidence intervals (CI). The association between each risk factor and the outcome variable was assessed using the Chi-square (2) test. For all analyses, statistical significance between variables was examined using P-value at critical probability of P < 0.05 (a p-value of less than 0.05 was taken as significant).

**Results**

Out of a total of 998 serum samples tested, 99 (9.9 %) reacted positive for brucellosis using the Rose Bengal Plate Test (RBPT). Bovine brucellosis was detected in nine out of the ten sectors in Nyagatare, and 62 herds out of the 205 herds studied were seropositive. The overall brucellosis herd prevalence rate (HP), i.e. at least one positive RBT reactor identified in a herd, was associated with sector ($X^2 = 8851.228$, P = 0.000), Breed ($X^2 = 413.567$, P= 0.002), and parity of the cow ($X^2 = 580.292$, P = 0.000).

Significantly higher HP values were reported for Byera (100%), Katabagemu (45.45 %), and Rwimbogo (42.86 %) sectors (Table 1). In Gatunda sector, while all the two herds from Byera Sector were seropositive giving an HP of 100 %. HP in other sectors ranged from 0 % to 33.33 % (mean 15.46 % ±11.35) in Karama sector, 7 % to 60 % (mean 32.18
± 13.21) in Karangazi sector, 0 % to 41 % (mean 35.49 % +/- 8.920) in Rwimiyaga sector, and, 0 % to 100 % (mean 18.41 % ± 26.60) in Tabagwe sector. The herd prevalence was 29.62% in Ankole cattle (95% CI: 28.36 to 30.87) and 23.71 (95% CI: 17.23 to 30.19) in purebred Friesian-Holstein cattle, with a statistically significant difference (x2 = 413.567, P = 0.000).

Individual animal prevalence (IAP), i.e. number of individual positive reactors, differed (P < 0.05) between and within the sectors. Significant higher overall IAP’s were found in Byera (20 %), Rwimiyaga (12.17%), and Rwimbogo (12.00 %) (Table 1). This study showed a higher seroprevalence (by RBT) of brucellosis in local cows than purebred Friesian Holstein cows (Table 2). Individual animal prevalence was 9.75 % (95% CI: 9.34 to 10.16) in Ankole cattle and 7.15 % (95% CI: 5.46 to 8.84) in Purebred Friesian-Holstein cattle with a statistically significant difference (x2 = 335.339, 28 df, P = 0.000).

There was no statistically significant difference in individual prevalence between Ankole cows and crossbred cows, Ankole and purebred Jersey, and Ankole and purebred Guernsey cattle (Table 2). Similarly, the prevalence of brucellosis in cattle was found to be higher in the older parities than younger ones. Overall seropositivity to bovine brucellosis was 5.9 % (12/204) for parity 1, 11.05 % (20/181) parity 2, 11.04 % (18/163) parity 3, 14.29 % (11/77) parity 4, and 8.82 % (3/34) for parity 5. However, no statistically significant difference was observed in the prevalence of brucellosis between male and female animals.

Table 1. Herd, within herd, and individual animal prevalence based on RBT stratified by sector in Nyagatare

<table>
<thead>
<tr>
<th>N1 +ve herds</th>
<th>Herd prevalence in % (CI)</th>
<th>Within Herd prevalence in % (CI)</th>
<th>N2: Cases Individual prevalence in % (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Byera 2</td>
<td>100.00a (100.0, 100.0)</td>
<td>20.00a (20.00, 20.00)</td>
<td>10 2 20.00a (20.00, 20.00)</td>
</tr>
<tr>
<td>Gatunda 3</td>
<td>0.00b (0.00, 0.00)</td>
<td>0.00b (0.00, 0.00)</td>
<td>0 0 0.00b</td>
</tr>
<tr>
<td>Karama 15</td>
<td>15.46c (15.46, 15.46)</td>
<td>11.53c (7.01, 16.04)</td>
<td>59 7 11.86c (11.86, 11.86)</td>
</tr>
<tr>
<td>Karangazi 81</td>
<td>32.18d (30.92, 33.44)</td>
<td>9.84d (9.38, 10.30)</td>
<td>427 42 9.84d (9.38, 10.30)</td>
</tr>
<tr>
<td>Katabagemu 11</td>
<td>45.45e (45.45, 45.45)</td>
<td>11.53ab (11.53, 11.53)</td>
<td>59 7 11.86c (11.86, 11.86)</td>
</tr>
<tr>
<td>Matimba 8</td>
<td>12.50c (12.50, 12.50)</td>
<td>1.89c (7.01, 16.04)</td>
<td>59 7 11.86c (11.86, 11.86)</td>
</tr>
</tbody>
</table>
Table 2. Herd, within herd, and individual animal prevalence based on RBT stratified by breed in Nyagatare.

<table>
<thead>
<tr>
<th>Breed</th>
<th>N</th>
<th>Cases</th>
<th>Herd prevalence in % (CI)</th>
<th>Within-Herd prevalence in % (CI)</th>
<th>Individual Prevalence in % (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankole</td>
<td>520</td>
<td>52</td>
<td>29.62 (9.75a, 11.19)</td>
<td>9.75a (9.34, 10.16)</td>
<td>8.88ab (8.32, 9.49)</td>
</tr>
<tr>
<td>Friesian</td>
<td>53</td>
<td>3</td>
<td>23.71b (3.01, 7.15b)</td>
<td>4.03 (1.76, 6.29)</td>
<td>6.67 (6.67, 10.16)</td>
</tr>
<tr>
<td>Crosses</td>
<td>248</td>
<td>22</td>
<td>33.16c (25.34, 8.42)</td>
<td>8.42 (6.67, 10.16)</td>
<td>9.50a (8.09, 10.09)</td>
</tr>
<tr>
<td>Jersey</td>
<td>12</td>
<td>1</td>
<td>28.89 ac (-10.40, 6.89)</td>
<td>6.11 (-3.89, 16.11)</td>
<td>8.88ab (8.32, 9.49)</td>
</tr>
<tr>
<td>Guernsey</td>
<td>13</td>
<td>1</td>
<td>31.69 ac (-16.25, 79.63)</td>
<td>4.00 (-7.11, 15.11)</td>
<td>7.17ab (-2.18, 16.53)</td>
</tr>
</tbody>
</table>

Total 832 30.30 9.83 9.49 9.92 (9.62, 10.22)

Figures with similar superscripts within a column are not statistically different at P < 0.05.

Discussion

The serological prevalence of brucellosis for 6 out of the 9 infected sectors of Nyagatare district included in this survey was around 10% with a 99% confidence. Considering that no formal control programme is in place, that about 1.1% vaccinated their cattle against brucellosis each year (6), and that other surveys in East African and sub-Saharan Africa frequently encountered prevalence in excess of 10% (31), the high prevalence of the disease here, is not surprising. Overall, our results indicated that bovine brucellosis was endemic in Nyagatare district.

Since very few vaccinations against brucellosis are carried out in the district, the seroprevalence figures obtained are a reliable estimate of exposure to wild type Brucella spp. The mean prevalence of the disease ranged from 1.89% in Matimba sector to 20% in Byera sector (Table 1). The finding of such a higher prevalence of the disease among the sectors is supported by observations of high incidences of abortion and retained placenta...
previously reported in Nyagatare (6).
The observed significant difference in herd, within herd, and individual animal prevalence of brucellosis signifies differences in breeds of dairy cows kept and animal husbandry practices prevailing among the different sectors. For example, 18 out of the 54 animals tested in Tabagwe sector (individual prevalence 5.56%) were improved breeds while 55 out of the 59 tested in Katabagemu sector (individual prevalence 11.96%) were crosses between exotic and local cows. The low prevalence in herds with a higher proportion of improved breeds is likely to be explained by zero grazing feeding practices that minimizes contacts between herds and animals. Most purebred cows are concentrated along the peri-urban centers, townships, and are mostly fed barna grass through the cut and carry system (6). However, the “cut and carry” system of feeding may serve as a potential risk for bovine brucellosis when the fodder is collected from areas used by indigenous traditional cattle.
The higher prevalence of bovine brucellosis in herds with a higher proportion of local or cross breed breeds of cattle is likely to be explained by the extensive system of grazing management practiced for such cows. In extensive grazing, animals from different locations, and likely of different brucellosis status, come into close contact in pastures or at watering points which facilitates spreading of the disease between and within herds (16, 17). Discharges from aborting animals or following normal birth contaminate pastures and possibly lead to higher herd prevalence rates in extensively managed animals. In addition, the mixing of local and exotic herds favors increased spreading of brucellosis between and within herds (26, 27). The prevalence of brucellosis in cattle in the extensive management system in this study agrees with reports from other countries with similar cattle husbandry systems (15, 4, 19, 26, 27).

It was observed that the local Ankole cattle or their crosses with exotic breeds made up the majority of the total sampled and also the sero-positive animals, hence, breed alone may not have played a key role in the results reported. As explained above, the breed factor relates more to how the breeds are perceived and managed rather differences in breed susceptibility to brucellosis per se. It can be argued that the observed differences in prevalence of brucellosis between indigenous and exotic animals are mostly attributed to differences in exposure to infectious animals or materials as a result of differences in management. The finding of high proportions of seropositive animals in indigenous as apposed to exotic breeds conform to results of a recent study in Tanzania which also reported similar observations (18).

There was 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 by OIE (24). However, despite this limitation, this study has revealed that, in spite of the fact that official data from Rwanda about brucellosis is lacking, the disease is still enzootic in some parts of the country and the risk posed to the human population and the economy of cattle production should not be underestimated (11).

In this discussion, parity was taken as a rough estimate of age and it appears a statistically significant effect of parity on prevalence of brucellosis existed. The prevalence of brucellosis increased with increasing parity or age of the cow. Susceptibility of older cows has been attributed to the effects of sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms. These substances tend to increase in concentration with age and sexual maturity of cattle (30). The observations that
the prevalence of brucellosis increases with parity or age of the cow are consistent with the
findings of several other researchers who reported significantly higher proportion of
positive reactors in older animals (29, 3, 34, 21).
In the present study, no male reactors were identified. None of the bulls tested in
the present study reacted positively to RBPT. However, the absence of male reactor
animals in this study could probably be due to the smaller number of male (n = 24)
animals studied as compared to females (n = 974). Even though it is difficult to draw a
firm conclusion, due to the smaller sample of males, the lack of difference between the
two sexes observed in this study corroborates established facts about the disease. Hirsh
and Zee (14) have reported that male animals are less susceptible to Brucella infection,
due to the absence of erythritol. Further, testes of infected male animals do not always
react to the infection or show low antibody titers (23, 10), thereby contributing to low
seroprevalence in this particular sex. Present observations are comparable with many
others (3, 4, 21).
In estimating exposure to brucellosis, the Rose Bengal Test (RBPT) should ideally
be used as a screening test, followed by more specific tests such as Serum agglutination
(SAT) or compliment fixation test (CFT) because the specificity of this test is low (20). In
addition, RBPT has limitations in the diagnosis of chronic brucellosis because the test
mainly detects IgM, yet the amount of IgM in serum of infected animals declines with
time to levels below the sensitivity of this test (34). However, these more specific tests are
currently not available in Rwanda. Nevertheless, regardless of low specificity, RBPT is an
excellent test to use in order to detect early infections (11). Therefore, it’s possible the
prevalence reported herein maybe an underestimation of the true situation on the ground.
Despite the dairy productivity implications, the high prevalence of brucellosis as
observed in this study pauses undisputable risk to the human population given the fast
growing dairy farming sector and intensification of livestock production in Nyagatare. The
high prevalence of bovine brucellosis, a livestock and zoonotic disease, which is easily
amenable to control through effective use of existing disease control technology such as
use reliable vaccines reflects failure of veterinary extension within the country.

**Conclusion**

From this study, it can be concluded that brucellosis is enzootic in Nyagatare and
could be the major cause of reproductive wastage previously reported from the same
district (6). This disease presents a significant impediment to the economic potential of
dairy production and is a zoonotic hence preventive and control measures should
immediately and strictly be implemented to protect animals and humans from brucellosis.
Further significance of the present findings relate to the fact that brucellosis is a
significant health hazard in human beings, causing a variety of chronic debilitating
illnesses for people who either come into contact with infected animals or consume
infected dairy products. Both, the control of infertility and prevention of brucellosis
infection in humans provide enough justification for the advocacy of brucellosis control
measures.

**Recommendations**
The authors recommend further epidemiological studies and isolation and
identification of the biotypes of Brucella responsible for infection in Nyagatare. Such
investigations have important implications for the type of vaccine that should be used and
when monitoring the efficacy of control programmes. The further investigations above
could should pave the way for mass vaccination to reduce the incidence of the disease to
significantly low and manageable levels prior to implementing a test and slaughter policy
where cattle, sheep and goat testing positive for brucellosis are slaughtered to remove source of infection from the herd.

Large-scale studies are also required to determine the epidemiology of brucellosis in humans. The impacts of the disease on the health of the local population can be decreased through awareness campaigns which can be initiated through training of animal health technicians on the routes of infection and preventive measures such as boiling of milk before consumption and avoidance of contact with aborted material and placentas. Owing to the relatively nonspecific symptoms in humans and a frequent lack of information on zoonotic diseases (9), it is further important to inform and collaborate with the human health services to increase the likelihood of correct diagnosis and treatment as well as to advocate the prevention of the disease through precautionary measures.

**Acknowledgements**
The authors thank the staff of Umutara Polytechnic and ISAR involved in this survey and the stock owners for their cooperation. Without their assistance the work could not have been carried out.

**References**


