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## The use of the milk ring test and rose bengal test in brucellosis control and eradication in Nigeria

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### ABSTRACT

In this study, milk and blood samples collected simultaneously from 532 trade cows to be slaughtered at Bodija abattoir, Ibadan (southwestern, Nigeria) were examined for antibodies to *Brucella* using the milk ring test (MRT) and the rose bengal test (RBT). Overall, 18.61 % of the milk samples were positive according to the MRT, while 9.77 % of the serum samples were positive according to the RBT. The difference was highly significant (Chi-square value 16.33;  $P < 0.05$ ); only 32 (6.02 %) of the samples were positive for both tests. The Red Bororo breed of cattle and the White Fulani had the highest positive rates, namely 20.93 % and 11.69 % for the MRT and RBT respectively. No conclusion can be drawn about sensitivity because we do not know the true status of the animals tested. It is, however, obvious that although the MRT and RBT are 1st-line screening tests for brucellosis in cows in some countries, their lack of specificity is of concern. Therefore, the requirement for other confirmatory tests that are more specific should be considered for control and eradication of the disease, especially in Nigeria.

**Key words:** brucellosis, cows, diagnosis, serology, zoonosis.

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### INTRODUCTION

Brucellosis is a disease of domestic animals with serious zoonotic implications in humans, causing huge economic losses to the livestock industry. Despite the preventive and control measures that exist in developing countries, there is still a high potential for transmission and spread of *Brucella* spp. via animals and their products imported from these countries<sup>1</sup>.

Serological tests are widely used for the diagnosis of brucellosis and they are almost exclusively used in eradication programmes<sup>11</sup>. Specific antibodies to *Brucella* in serum and other body fluids are detected by an ever-increasing variety of techniques; some of the tests have been developed with a view to differentiating antibodies resulting from infection and those from vaccination, and others aim to detect the chronic carrier animal in areas or herds with a very low incidence<sup>11</sup>. Body fluids such as serum, uterine discharge,

vaginal mucus, milk, or semen from suspected cattle may contain different quantities of antibodies of the IgM, IgG1, IgG2 and IgA types directed against *Brucella*<sup>3</sup>. Because infected cattle may or may not produce all antibody types in detectable quantities, several tests are used to detect brucellosis. The commonly used blood tests are the complement fixation test (CFT), rose bengal test (RBT), serum agglutination test (SAT) and the enzyme-linked immunosorbent assay (ELISA). Less often performed are the anti-globulin (Coombs) test and the 2-mercaptoethanol or rivanol adaptations of the SAT. The milk ring test (MRT) and ELISAs are available for detecting antibodies to *Brucella* infection in milk and the MRT test is cheap, simple and requires no specialised equipment to perform. It detects anti-*Brucella* IgM and IgA bound to milk fat globules. However, false positive reactions occur when milk that contains colostrum or milk at the end of the lactation period is used. Also, milk from cows suffering from a hormonal disorder or cows with mastitis may produce false reactions<sup>5</sup>. The test may be insufficiently sensitive to detect antibodies in milk that contains low concentrations of IgM and IgA or that lacks the fat-

clustering factors<sup>9,12,13</sup>. Because antibodies in milk rapidly decline after abortion or parturition, the reliability of the MRT in individual cattle or in bulk tank milk is reduced<sup>8</sup>.

The RBT is a simple agglutination technique. Because the test does not need special laboratory facilities and is simple and easy to perform, it is used to screen sera for antibodies to *Brucella*. The test may yield false negative results, although rarely in infected cattle that give positive results with the CFT<sup>13</sup>. Although the low pH (+3.6) of the antigen enhances the specificity of the test, the temperature of the antigen and the ambient temperature at which the reaction takes place may influence the sensitivity and specificity<sup>10</sup>.

This study was aimed at evaluating the ability of the MRT and RBT to detect antibodies produced in suspected infected animals with the possibility of using them in brucellosis control and eradication programmes. These tests were chosen because they are less cumbersome to perform on a large scale and/or require no special equipment and expertise compared with other commonly used assays such as CFT or ELISA.

### MATERIALS AND METHODS

#### Location

The Bodija municipal abattoir in Ibadan, southwestern Nigeria, was used for this study. This abattoir was chosen because over the years it has recorded an average of 6.00 % prevalence of bovine brucellosis in the population of slaughtered cattle<sup>6</sup>. The choice of cows from which samples were collected was based on cooperation of animal owners and the inclusion criteria (*i.e.* lactating animals that are apparently healthy). The study was conducted over a period of 4 months during which we believed we would have screened a representative number of animals based on our inclusion criteria.

#### Sample collection and handling

Milk samples of approximately 10 ml were collected in sterile universal tubes from each of the 532 cows prior to slaughter and approximately 10 ml of blood was

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collected in 15-ml sterile tubes from each of these animals during slaughter. The breed and age of each cow were recorded. Milk samples obtained from the animals were kept refrigerated at 4 °C overnight prior to examination by MRT<sup>2</sup>. The blood samples were allowed to clot and centrifuged at 3000 g for 5 minutes. Serum samples were decanted and stored at -20 °C until they were assayed. The serum samples were examined by RBT<sup>2</sup>.

## RESULTS

Ninety-nine (18.61 %) and 52 (9.77 %) of the 532 milk and serum samples screened were positive according to the MRT and RBT, respectively. Thirty-two (6.02 %) of the samples were positive in both tests. The highest positive rate of 20.93 % was obtained among the Red Bororo breed of cattle using the MRT, while the White Fulani breed had the highest infection rate of 11.69 % in the RBT (Table 1). The young adult age group (1–3 years) and the adult (>3 years) cattle had the highest infection rates of 20.96 % and 9.86 % with the MRT and RBT, respectively (Table 2).

## DISCUSSION

The significantly high difference (chi-square 16.33,  $P < 0.05$ ) in the proportions of samples positive according to the MRT and the RBT indicates that, although these 2 tests are generally used for screening, especially in developing countries, other tests are required for confirmatory diagnosis. This is in agreement with Morgan<sup>11</sup>, who stated that the tests should be used in conjunction with the established tests and not instead of them. According to Morgan<sup>11</sup> and Bercovich and Moerman<sup>5</sup>, the higher proportion of positives by the MRT might result from false positives, which could be due to many causes, including mastitis, colostrum, collection at the end of the lactation period, or a hormonal disorder. Although care was taken to avoid taking samples from animals with mastitis during this study, it was not impossible that some cases would have been included, which would give a false positive result. Since the milk samples were collected without a history of parturition status, it is likely that milk in one or more of these adverse states was included in the study. As health records from all the animals were not available, it is difficult to ascertain the condition of the animal at slaughter. It has been shown that different serological tests used in the diagnosis of brucellosis vary considerably in their ability to detect antibodies of a particular immunoglobulin class<sup>4</sup>. Infected animals may or may not produce all antibody isotypes in detectable quantities. Therefore, since the capacity

Table 1: Results of the RBT and MRT of the animals sampled based on breed.

Breed	Number screened	No. positive (MRT) (%)	No. positive (RBT) (%)
White Fulani	231	43 (18.61)	27 (11.69)
Red Bororo	86	18 (20.93)	6 (6.98)
Kuri	47	9 (19.15)	5 (10.64)
Sokoto Gudali	89	13 (14.61)	5 (5.62)
Mixed	79	16 (20.25)	9 (11.39)
Total	532	99 (18.61)	52 (9.77)

Table 2: Results of the RBT and MRT of the animals sampled based on the age group.

Age	Number screened	No. positive (MRT) (%)	No. positive (RBT) (%)
>3 yrs	365	64 (17.53)	36 (9.86)
1–3 yrs	167	35 (20.96)	16 (9.58)
Total	532	99 (18.61)	52 (9.77)

of serological tests to reliably detect brucellosis depends on the presence of detectable antibodies at the time of examination, some infected animals will inevitably elude detection<sup>7</sup>. This may explain the relatively lower number of positive cases detected by the RBT in our study, since detection of antibody is based on the IgG class. Since most animals slaughtered in this abattoir come from unregulated markets in the northern parts of Nigeria and neighbouring African countries, it is difficult to establish their vaccination status. However, we know that owing to limited patronage of veterinary services by most of these livestock owners, routine vaccination against brucellosis is not readily carried out in Nigeria, so that our results were probably not affected by vaccination.

In general, the RBT and MRT have been shown in other studies to have high sensitivity but lower specificity. The MRT is not normally used on individual animals because of the higher rate of false positives (less specificity).

Based on the outcome of this study, it is suggested that, although MRT and RBT are generally useful for screening for brucellosis, especially in developing countries where other tests are cumbersome to perform on a large scale and/or require special equipment and expertise, these tests still have major limitations where vaccination or medical records are not available. As a result of these limitations, other confirmatory tests e.g. ELISA, CFT, SAT must be carried out in conjunction with MRT and RBT in order to confirm the brucellosis status of trade cattle slaughtered in the Nigerian abattoirs. The ELISA is an available assay for use on milk and serum and is very useful where large numbers of samples require testing.

The milk ELISA is used on pooled samples, which is more cost-effective than testing individual animals. Although these tests may be very expensive, they are needed to confirm the brucellosis status of animals slaughtered in our abattoirs in order to safeguard the health of the general public and in particular that of the people directly involved in the business of meat inspection and processing.

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