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Research Article

Prevalence of Brucellosis in Cattle Slaughtered in the Municipal Slaughterhouse of Maputo City and Magude District (Mozambique) in 2015

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Abstract

Brucellosis is an infectious disease caused by bacteria of the genus *Brucella* that infect animals and humans, always presenting a great economic and public health impact, with regional asymmetry, associated with the raising and trade of cattle, especially in developing countries. It is a worldwide disease and is endemic in Mozambique. The present study aimed to determine the prevalence of brucellosis in slaughtered cattle in Maputo city and province, in this way 340 blood samples of male and female cattle in reproductive age from different parts of the country were collected. The sera obtained from the samples were submitted to the screening test, Bengal Rose (BR) and the Complement Fixation Reaction (CRF) test for confirmation, and later seropositive were subjected to the i-ELISA test. Overall seroprevalence was 16.5% (56/340); and differed significantly between females [19.7% (29/147)] and males [13.9% (27/193)]. The results obtained show a high seroprevalence of brucellosis in slaughtered animals and the potential risk of transmission of the disease to workers, which may cause serious public health problems, requiring the implementation of a health education program for slaughterhouse workers as well as their managers.

Keywords: Cattle; Brucellosis; Slaughterhouses; Public Health

Introduction

Brucellosis is a zoonosis that causes major health problems and has serious economic damage, especially in rural areas where cattle breeders and herds are concentrated [1]. According to Martins [2], *Brucella* can remain viable in meat for months because it is little affected by muscle acidification, especially when carcasses are kept at refrigerating or freezing temperatures, also surviving brine and salting. In meat products, *Brucella's* survival depends on the technological process employed and the degree of meat contamination at the beginning of the process. As in milk, subjecting the meat to heat treatment is essential, as is strict hygiene measures during carcass slaughter and evisceration. The disease has a high morbidity rate and low mortality; however it is a health problem for professional groups such as veterinarians, employees of dairy farms and slaughterhouse workers. In cattle, brucellosis is a disease caused by *Brucella abortus* that produces reproductive changes in animals [3].

Mozambique has optimum conditions for beef cattle farming and is divided into eleven provinces with 3 main regions (south, center and north), the province of Maputo located in the southern region stands out in the beef cattle farming. In Mozambique, the first clinical suspicion of brucellosis in cattle dates back to 1942, and may be remote, as it was screened in South Africa in 1913 and hence probably spread to Mozambique [4]. According to Abreu [5], *B. abortus* was isolated for the first time in 1951, and the following

year, the southern zone of Save was considered as an area infected by the disease and the first live B19 vaccines were applied [4]. This disease is a serious problem to National livestock activity, although the actual prevalence in the country is still unknown [6,7]. The present study was carried out at the municipal slaughterhouse of Maputo city and the Magude-Sede district in Mozambique, from September to November 2015, to determine the seroprevalence of brucellosis in cattle slaughtered at these sites. The municipal slaughterhouse of Maputo city is under private management and has workers distributed at various stages of slaughtering and carcass preparation operations (stunning, sticking, skinning, evisceration, quartering, washing, stowage), and a livestock (official inspector) who inspects the carcasses. The animals slaughtered come from different parts of the country, with the highest incidence from the provinces of Tete, Gaza, Manica and Inhambane. Magude-Sede is one of the districts of Maputo province that holds the largest cattle herd, owns a slaughter house and meat processing. The workers are distributed in various stages and operations of slaughter and preparation of carcasses (stunning, sticking, skinning, evisceration, quartering, washing, stowage). These beef slaughtering and processing units have sanitary inspection and are authorized by National Direction for Veterinary Services (DNSV)/ Provincial Liver stock Services (SPP) and Ministry of Health (MISAU) to perform the slaughter and dispatch of meat only and internal consumption in accordance with the rules prescribed in the Animal Health Regulation [8].

Material and Methods

Study population and sampling

Based on a systematic probability sampling (K: 2), twice a week, 4 weeks a month and 3 months of the study period were selected at the time of slaughter male and female of reproductive age of the bovine species. The sample size was calculated assuming an expected prevalence of 50%, with a significance level of 5%, using the following formula [9]:

$$n = \frac{1,96^{2} p_{exp} \left(1 - p_{exp}\right)}{d^{2}}$$

Where: **n** is the sample size, $\mathbf{P}_{exp}$ is the expected prevalence and $\mathbf{d}^2$ is the precision level. Subsequently the sample was adjusted for the slaughtered bovine population by the following formula:

$$n_{cor} = \frac{N * n}{N + n}$$

Maputo city municipal slaughterhouse

The municipal slaughterhouse received monthly an average of 90 to 120 animals. Based on the average slaughter was estimated number of bovine population during the 3 months of study (330 animals), from which the sample size of 178 animals was calculated.

Magude - sede district slaughterhouse

It received a monthly average of 280 animals from different Administrative Posts in the district for slaughter. From this animal population a sample of 162 was estimated.

Collection and conditioning of blood samples

Blood samples were collected in vacutainer tubes without anticoagulant, at the time of bleeding into the neck vessels for further extraction of the serum. The tubes were previously identified with the order number of the tubes and a sample identification form containing information regarding the date of collection, order number, sex and origin of the slaughtered animal.

The samples were then packaged and stored in isothermal boxes containing ice packs and then transported to the Mozambican Agricultural Research Institute (IIAM) where they were processed at the Central Veterinary Laboratory of the Animal Sciences Directorate (LCV/DCA). Samples were stored at 4oC until processed. The samples were processed within 48 hours of collection, where they were centrifuged at a speed of 3,000 rpm for 10 minutes in a centrifuge (Centrifuge Baird and Tatlock Auto Bench Make centrifuge) to obtain the serum. Subsequently the supernatant (serum) of each sample was pipetted into previously identified 1.5 ml Eppendorf tubes and stored at -20oC freezing until serological testing [10].

Laboratory tests

Serum samples were initially submitted to the Bengal Rose Test (BRT). The BRT positive were subjected to the Complement Fixation Reaction Test (CFR) for confirmation. The serum that tested positive on both tests were considered to be really positive. However, for the differentiation of vaccine antibodies from those caused by natural infection, the i-ELISA test (ID Screen® Brucellosis Serum Indirect Multi-species) was used, which has the advantages of greater specificity. The i-ELISA was processed according to the manufacturer's recommendations.

Bengal rose test

The test was performed as described in the Onderstepoort Veterinary Research Institute Manual in the Republic of South Africa (1996) and consisted of the addition of 30µl of serum and one drop of acidified Bengal Rose antigen on a unitex plate containing divisions. In each space was placed the drop of serum to be tested and the antigen, and then the plate was gently shaken, applying rotary movements for 4 minutes, after which time was taken the reading based on the occurrence or not of agglutination. Sera with any degree of agglutination were considered positive.

Complement fixation reaction test

The test was performed according to the procedures described in the National Institute for Veterinary Research (INIVE) [11] brucellosis diagnosis manual. The antigen used was produced by the Onderstepoort Veterinary Research Institute (RSA). As a complement, *Cavia porcellus* serum (guinea pig), used in the hemolytic system formed by hemolysin-sensitized *Ovis aries* red blood cells (rabbit antibody against sheep red blood cells) titrated as recommended by INIVE [11]. To perform the test the serum was initially inactivated in a water bath at 60oC for 1h. This was followed by the preparation of the complement obtained from guinea pigs and hemolysin. The test was performed in 3 test tubes, 2 first for dilutions (1:5 and 1:10) and the last for control of serum anti-complementary activity. In the tubes arranged in a row, 0.4 ml of physiological solution (0.85% NaCl) was deposited in the first and last tube and 0.25 ml in the second. Then 0.1 ml of inactivated serum was added to the first and third tubes, and homogenized. After that 0.25 ml of the concentrate was extracted from the first to the second after mixing, 0.25 ml was discarded, thereby obtaining dilutions 1: 5 in the first tube and 1:10 in the second. Subsequently, the mixture was quenched in a 60°C water bath for about 30 minutes and allowed to cool. Then 0.25 ml of the standard antigen was added to the first 2 tubes and 0.5 ml of the title obtained (1:57) in all tubes and incubated in a 37°C water bath for 30 minutes. Finally, 0.5 ml of the hemolytic system was added to all tubes and incubated in a 37°C water bath for 30 minutes. It was allowed to stand for 30 minutes and the first reading was made and then kept at 4°C for 18-24 hours, after which the last reading was made based on the degree of hemolysis.

Indirect ELISA test (i-ELISA)

The indirect ELISA kit prepared by Innovative Diagnostics (ID-vet) was used for direct detection of antibodies against *Brucella abortus, melitensis* and *suis* in serum and plasma for individual or group samples. The test was performed according to the manufacturer's recommendations.

Statistical analysis

Serological testing results were entered into a database in Microsoft Office Excel 2007 and analyzed using the SPSS statistical package (version 20). Seroprevalence was calculated by the proportion of positive sera in relation to the total sera tested. Seroprevalence was compared according to sex, age and provenance of the animals using the Z test to compare proportions. For all analyzes, a significance level of 5% was considered.

Results and Discussion

A total of 340 animals were selected for the study, of which 178 were from Municipal slaughterhouse of Maputo city and 162 from district of Magude-Sede. Of the total number of study animals, more than half (55.6% in Municipal de Maputo and 58.0% in Magude -Sede) were male (Table 1) and all females had no iron mark or vaccination record sheet. brucellosis or relevant health information.

	Sex		
Slaughterhouses	**Male**	**Female**	**Total**
Magude-Sede	94 (58%)	68 (42%)	162 (100%)
Municipal de Maputo	99 (55,6%)	79 (44,4%)	178 (100%)
Total	193 (56,8%)	147 (43,2%)	340 (100%)

Table 1: Distribution of animals sampled by slaughterhouse and sex.

Brucellosis evaluation in animals

It was observed that 17.4% (31/178) of the animals slaughtered at the Maputo municipal slaughterhouse were serologically positive for *B. abortus*, based on the screening test (Bengal Rose) as well as the confirmatory test (Complement Fixation Reaction), while at Magude-Sede slaughterhouse a seroprevalence of 15.4% (25/162) of slaughtered animals was found (Table 2).

Slaughterhouses		***BR***			***CFR***	
	T	**R**	**%**	**T**	**R**	**%**
Magude-Sede	162	25	15,4	162	25	15,4
Municipal de Maputo	178	31	17,4	178	31	17,4
Total	340	56	16,5	340	56	16,5

Table 2. Frequency of positive samples from serological tests by provenance.

T: Total Animals Sampled; R: Reactive Animals, %: Percentage (Prevalence).

The Municipal slaughterhouse of Maputo city is the largest cattle slaughtering center and therefore the largest meat consumption area at National level. The found seroprevalence of 17.4% illustrates the health status of animals slaughtered in this establishment under the same circumstances as healthy animals, endangering slaughterhouse workers and consumers. On the other hand, seroprevalence of 15.4% was found in animals coming from the different Administrative Posts for slaughter at the Magude-Sede slaughterhouse. This result, although lower than 17.7% found by Chilengue [12], also represents a potential risk of *B. abortus* transmission beyond its importance for individual occupational health, and has great relevance to public health, as slaughterhouse workers are the first hosts to be exposed to the etiologic agents of zoonoses [13]. However, the meat of most animals slaughtered is shipped to the large consumption areas of Maputo city.

The overall seroprevalence found was 16.5% (56/340) (Table 2), this result is within the National parameter ranging from 6.6 to 20.3% [14], it is above the seroprevalence range found by Manhiça [15] in southern Mozambique ranging from 6.1 to 10.3%, and 13% seroprevalence in beef cattle found by Pereira (1997).

Studies conducted in some countries of the African continent, such as Angola, Nigeria, Egypt and Zimbabwe [16-18], reported lower seroprevalence (5 to 9.9%) compared to that found in the present work. However, the comparison of these values deserves attention, since the methodology applied was different, especially regarding the serological tests used. In the present study, BRT and CFR seropositive samples were submitted to the subsequent i-ELISA (ID Screen® Brucellosis Serum Indirect Multi-species) test, which was used to confirm and detect antibodies against *Brucella spp*., where it was found that the seroprevalence recorded in the first two tests resulted from natural infection. According to Dias [19], contamination of meat occurs most often at the time of slaughter and evisceration of animals, at which time contaminated material such as blood, bone marrow or feces can dirty the carcasses if there is no increased hygienic care. On the other hand, this disease has negative effects on reproduction due to its association with abortions and may reflect animal management problems and lack of vaccination. Previous studies have shown a higher frequency of animals reacting in herds that did not practice animal vaccination [20-22].

Of the total cattle sampled in both slaughterhouses, 43.2% (147/340) were female and 56.7% (193/340) were male. The results of brucellosis seroprevalence as a function of sex are presented in table 3. The seroprevalence of brucellosis in females (19.7%) was higher compared to seroprevalence in males (13.9%), however the difference is not statistically significant (P > 0.05). These results corroborate those reported in other studies [23,24], which also observed that females were more affected than males.

	Male			Female			Total		
Slaughterhouses	**T**	**Positive**	**%**	**T**	**Positive**	**%**	**T**	**Positive**	**%**
Magude-Sede	94	12	12,7	68	13	19,1	162	25	15,4
Municipal de Maputo	99	15	15,1	79	16	20,2	178	31	17,4
Total	193	27	13,9	147	29	19,7	340	56	16,5

Table 3: Result of seroprevalence by provenance and sex.

T: Total Animals Sampled; **%:** Percentage (Prevalence).

There was a higher percentage of affected females, which could compromise the herd evolution in the places of origin of the animals, considering that the disease compromises the reproductive system, causing miscarriage in the last third of pregnancy and, in its chronic stage, can compromise the mammary gland. Males usually develop orchitis and epididymitis, consequently causing sterility [1].

The lack of a serologically positive animal control and sanitation program increases the risks of infection in humans, especially when they do not undergo sanitary slaughter. Mello [25], cited by Spinola., *et al*. [26], pay attention to this pathology as a social problem characterizing its damage to human groups and the economy of regions where it is endemic. In endemic areas where bovine brucellosis occurs there is a potential public health risk [27,28], because

infection in animals is chronic and persists for life. As brucellosis is not usually transmitted from one human to another, prophylaxis in humans is made by combating and eliminating disease in animals (positive reactors).

The prospecting actions carried out by the Provincial Livestock Services and National Directorate of Veterinary Services (SPP/DNSV) in the familiar and private cattle breeding sector in recent years shows the existence of brucellosis in cattle, but its prevalence is poorly known as well as its impact. economic. Data collected from the 2011 to 2014 annual reports in the IIAM serology section at DCA/LCA indicate animal disease prevalence values ranging from 6.6 to 10.2%, while DNSV surveys [29], indicate a variation of 3 to 28% of animals tested positive for brucellosis in the southern and central areas of the country.

Conclusion and Recommendations

The overall seroprevalence found in animals slaughtered at the municipal slaughterhouses of Maputo city and Magude-Sede district was 16.5%, can be considered high for the region, so there is a need to reinforce prophylaxis measures to avoid the risk of infecting slaughterhouse workers and consumers.

It is recommended to increase epidemiological surveillance of brucellosis in slaughterhouses through health Certification and testing of slaughter cattle. To implement control and prophylaxis measures contemplated in the National Program for the Prevention and Control of Brucellosis and Bovine Tuberculosis (PNPCBT).

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