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Research Article

Detection of Brucellosis in Blood of Cows in Some Farms of Khartoum State

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ABSTRACT
Brucellosis is an infectious disease that affect human as well as animals. The disease is caused by exposure to a bacterial species belong to the genus Brucella. Brucellosis has wide distribution in sub-Saharan African countries, including Sudan in which the diagnosis remains a great challenge and basically depend on serology. The present study was carried out on five dairy cattle farms allegedly free from brucellosis, but with sporadic cases of abortion. As well-known Sudan has a many dairy industries with peri-urban dairy establishments which built in order to improve milk availability to rural communities. The aim of this study is to estimate the prevalence of brucellosis in Sudan, using samples submitted to collect from different farms. Five farms were tested with Rose Bengal Test (RBT) and Enzyme-linked Immune-absorbent Assay (ELISA). Parallel testing was used to determine whether a farm was to be considered positive or not. The result showed that out of 70 cow's Blood samples examine 22 (31.4%) had positive RBPT results while the rest (68.6%) had Negative RBPT results. Of the Five farms which were examined the positive cows were observed in only 2 farms. The results show the need to carry out surveillance of brucellosis in cattle in Sudan to understand the spatial distribution of the disease in the country. These surveillance could reduce the risk of Brucellosis spreading and consequently improvement will take place in the quality of livestock and their dairy product which consumed by the human in those areas.

Key words: Brucellosis, Cows, Farms, Serology, Rose Bengal test.

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INTRODUCTION

Brucellosis is a worldwide recognized bacterial contagious zoonotic disease; it is cause of significant reproductive losses in livestock. Brucellosis is more frequently detected among developing and middle income countries, where the poor hygiene, consumption of raw animal products (like milk), and poor public health education programs are existed [1].

The etiological agent of *Brucellosis* is member of genus Brucella, The *Brucella* is Gram-Negative, facultative intracellular bacterium and have six species *Brucella melitenses, Brucella. abortus, Brucellasuis, Brucellaovis*,

Brucellacanis and *Brucella Neotomae* [2]. Bovine brucellosis is basically caused by *Brucella abortus*. However, *Brucella melitensis* may spill over from the few ruminant reservoirs and infect the cattle as well. On the other hand, *Brucellasuis* infects pigs, as well as humans [3]. Among cattle, *Brucella bortus* causes abortions and eventually lead to reduction in their fertility rate [4]. However, among humans, it can cause chronic infections including undulant fever, endocarditis, arthritis, and osteomyelitis [5].

Among animals, Brucellosis basically spread through contact with the infected placenta, fetus, fetal fluids and vaginal discharges. Bacteria could also be present in infected animals blood, urine, milk and semen and thus it may harbor greet risk of disease spreading [6]. Man is infected by animals Brucellosis through direct or indirectly by ingestion of animal product as well as by inhalation of air borne agents [7]. Brucellosis is usually an occupational disease because of the contagious capabilities of its causative agent. Thus most cases occur among veterinarians, farmers, hunters, and livestock producers. Sometimes infection occurs after drinking raw milk or eating unpasteurized cheese [8].

The socio-economic effects of animal brucellosis could be observed clearly in communities who relied on animal production as their livelihood. Losses in live stocks are occurring as result of offspring losing and these are due to abortion, stillbirth and infertility. Indirect losses are due to reduction in milk yields and humans suffering from the disease, Morbidity and Mortality rate is high and the disease spread quickly. RBPT detection of Antibody the test is screening test but may be over sensitive for diagnosis in individual animals particularly vaccinated ones and confirm the result by ELISA test detection of Antigens specific and sensitive. Only few data are available about the prevalence and incidence of animal Brucellosis in Sudan and most previous studies are focused on Human brucellosis. Therefore this study was aimed to determine the prevalence of Brucellosis among cows in farm in Khartoum state by using RBPT. Result obtained by this study may help the veterinarian to put polices to reduce the distribution of disease among livestock and thus to save more stock live which may lost due to brucellosis induced abortion.

MATERIALS AND METHODS

This study was Survey Study to Detect Brucella in blood of cows from different farms In Khartoum state. The study was conducted in five different farms in Khartoum state between the periods from September to December2019.

Study population

Cows from different farms were included in this study.

Sample size and sampling technique

Seventy serum samples were collect from farms. Then the serum samples, Blood were collected from cow by vein puncture of jugular veins using vacationer tube with needle holders. All blood samples were transferred into plain blood containers then centrifuged at 3000 RPM for 3 min to obtain sera.

Serological tests:

Rose Bengal Plate Test (RBPT):

The RBPT is one of group of tests known as the buffered Brucella antigen tests which rely on the principle that the ability of IgM antibody to bind to antigen is marked reduced at a low pH .The RBPT is simple spot agglutination test where drops of stained antigen and serum are mixed on plate and any resulting agglutination signifies a positive reaction. The test have an excellent screening capabilities, however it may be over sensitive for diagnosis vaccinated animals. All sera were tested using the Rose Bengal (RBT) according to the procedures described by the OIE [6]. For the test, the RBT antigen, control and test sera were brought to room temperature 30 min before use as homogeneous suspensions. A total volume of 25 µl were dispensed from both serum and antigen (Reagent) individually in an adjacent manner on agglutination plate consisting of 48 white tiles; mixed together rapidly and thoroughly then the plate was shaken lightly for 4min. The degree of agglutination reactions was recorded immediately under good light and with the naked eye with a cut off time of 4min, after which agglutinates revealed were not taken into consideration.

Enzyme-linked Immunosorbent Assay (ELISA) Test

The ELISA test offer excellent sensitivity and specificity whilst being robust fairly simple to perform with a minimum of equipment and readily available from a number of commercial sources in kit form .they are more suitable. Cow's sera were tested using a Brucella Antibody Test Kit (SVANOVIR BrucellaAb-I-ELISA) according to the instructions of the manufactures. Briefly, 50µl of the samples were added to each test well. Then, an amount of 50µl of controls was dispensed in duplicate test wells. A diluent of 1:100 of competing antibody which is stored at – 20 °C was prepared, and then 50µl of it was added into all test wells. After that the plates were covered with a protective foil and incubated for 1 h at 37 °C. The plates were washed four times with 300 µl per of the wash then dried completely by tapping the plates firmly on an absorbent paper, Then 100µl of conjugate (1:100).

Data collection

Structured questionnaire was used to ask the farmer's bout the cows, their breeding as well as the vaccination status.

Data analysis

Data was analyzed by computerized method (Statistical Package for Social Sciences) (SPSS version 20).

Ethical consideration

Approval was taken from research ethic committee of Al Zaeim Al Azhari University and verbal consent was taken from each farm, and all the information's taken were treated confidentially and was only be used for research purposes.

RESULTS

A total of 70 samples were tested from 5 local Farms (70 blood/serum samples). Rose Bengal test (RBPT) was applied to all collected samples, and 22 of the 70 cow's Blood samples (31.4%) had positive RBPT results and 48 of the 70 cow's Blood samples (68.6%) had Negative RBPT results (Figure 1). Similarly, all samples had RBT or diagnostic card test, were tested by ELISA. ELISA test was showed same result of RBPT (Table 1). we take the blood samples from cows living in five farms in Khartoum state and by comparison of farms we found that farm number III and IV were most affected farms with 100% infections rate followed by farm number five while farm number 1 and 2 showed no evidence of Brucellosis (Table 2). The result also show that cows in local breeding type was most affected group (19 out of 22) positive cases followed by cows in a hybrid type of breeding (1 out of 22) positive cases while the none of the cows in an international breeding positive for Brucellosis (Table 3). Furthermore our result reveals that none of the vaccinated cows was affected by Brucellosis while all non-vaccinated cows were affected (Table 4).

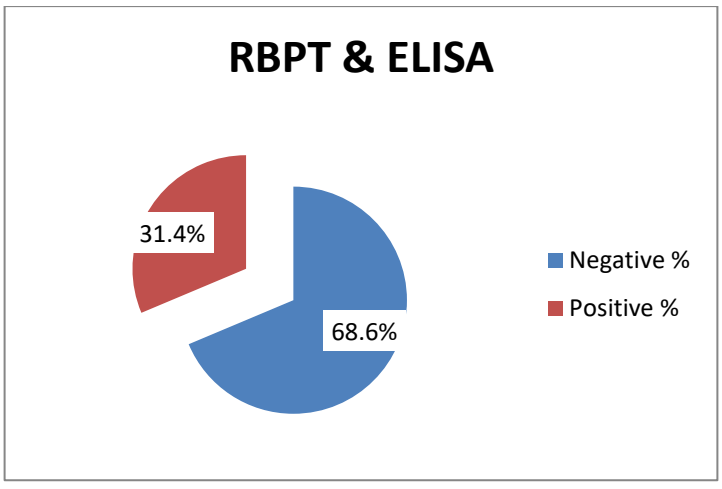

Figure 1: RBPT results for Brucellosis in the 5 Farms:

Table1: Percentages of the RBPT & ELISA results:

Test	Negative samples %	Positive samples %
RBPT	68.8 %	31.4 %
ELISA	68.8 %	31.4 %

Table 2: Percentages of the RBPT & ELISA results according to the farms

Farmers	Total number of cows , been examined	Number of positive samples by Serological tests (RBPT & ELISA)
I	17	0
II	17	0
II	8	8
V	14	14
V	14	01
Total	70	22

Table3: Percentages of the RBPT & ELISA results according to the cows breeding

Cows Breeding	Number of Negative samples by Serological tests (RBT & ELISA)	Number of positive samples by Serological tests (RBT & ELISA)
Local	28	19
International	12	2
Hybrid	8	1

Table4: Percentages of the RBPT & ELISA results according to vaccination

Vaccine	Number of Negative samples by Serological tests (RBT & ELISA)	Number of positive samples by Serological tests (RBT & ELISA)
Vaccinated	48	0
Non-Vaccinated	0	22

DISCUSSION

Brucellosis continues to be a problem in many regions in the world, led by developing countries especially (9). In this study, the prevalence of *Brucella* was investigated in bloods samples collected from bovine animals on five different farms. The collected blood samples had RBPT and ELISA test performed, respectively. Linked to the results of the two tests, 22 of the 70 cow's blood samples (31.4 %) were identified as suspicious. The result obtained in this study was similar to a previous study who had been the first attempt to estimate the prevalence of brucellosis in Zimbabwe as a whole, using samples submitted to the Central veterinary laboratory between 2010 and 2014 in which total of 156 farms were tested with Rose Bengal Test (RBT), Complement Fixation Test (CFT) and Milk Ring Test (MRT). Parallel testing was used to determine whether or not a farm was to be considered positive: 30.1% (95% C.I.: 23.5% - 37.8%) of the farms tested were found positive (47/156) (10).

On the other hand our study results was higher than that obtained by Upadhyay et al (2007) who conducted sero-surveillance in 17 randomly selected districts of Uttar Pradesh State, India. They recorded an overall prevalence rate of bovine brucellosis of (12.77%) By AB-ELISA (415 cattle were screened) (11). However, an old survey in the Gash and Tokar areas in Kassala Province (1968) for investigation of bovine brucellosis incidences and there was lower than that of our study, with prevalence rate in Gash an Tokar was (1.1 %), (5.5%) respectively (12).

Our study has some limitations. First, we did not evaluate knowledge, attitudes, and behaviors of participants on cow. Therefore, we could not discuss the risk factors that may affect the prevalence. Public health experts play an important role in the prevention of diseases linked to zoonosis and in the protection of public health.

With this aim, the WHO recommends that surveillance systems for food-sourced diseases should be strengthened and that data obtained from this surveillance be used in the planning, operation, and assessment of public health policies (13).

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