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Serological Detection of Brucellosis in Cattle and Human

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Abstract: The aim of present study was to investigate the seroprevalence of brucellosis among cattle and cattle-owners at selected area of Khartoum state. This research was carried out in Gubul Awliaa area farms, out of 175 unvaccinated cattle 84 (48%) were shown positive agglutination reaction towards *Br. melitensis* while 44 (26.4%) showed positive reaction toward *Br. abortus*. Mix infection was also reported. The highest incidence of infected animals and mix infection were recorded in cows with more than 10 years old. Cows milking between 6-10 times showed highest incidence of the infection. 33.1% of positive *Br. melitensis* cattle showed titre of 160 while 24.6% of the positive *Br. abortus* showed titre of 160. The Ring Milk Test (RMT) was positive in 80% of examined milk samples. On the other hand 40% from examined milker-sera showed positive agglutination results with *Br. melitensis* with titre of 160.

Key words: Ring Milk Test (RMT), *Brucella* spp., *Br. melitensis*, *Br. abortus*

INTRODUCTION

Brucellosis is a contagious bacterial disease of animals, which is transmitted to man (Al-Sekait, 1999). The disease is widely distributed and has been recorded in 120 out of the 175 (68.8%) countries of the world (Nielson and Duncan, 1990). The same authors added that bovine brucellosis was eradicated from Australia. In Africa bovine brucellosis has been reported in 44 of 49 African countries (Benin, Liberia, Equatorial Guinea, Mali and Morocco did not report), in Arab countries, the disease has been reported from all the Arab countries except Morocco (Seimenis *et al*., 2006). Brucellosis in cattle was reported in all parts of Sudan and the prevalence rate was found to be higher in cattle compared to other animal species (Mohud, 1989, El-Sharif, 1994; El-Ansary and Mohammed, 2001).

The etiological agents of the disease are members of genus *Brucella* (Rajashekara *et al*., 2006). Previously six species *Br. melitensis*, *Br. abortus*, *Br. suis*, *Br. ovis*, *Br. canis* and *Br. neotomae* were identified in the genus *Brucellae* (Rajashekara *et al*., 2006). However DNA-DNA hybridization studies have shown that only one species *B. melitensis* exist in the genus and the other species were actually biovars (Rajashekara *et al*., 2006).

Among animals, *Brucella* is usually transmitted by contact with the placenta, fetus, fetal fluids and vaginal discharges from infected animals. Animals are infected after either an abortion or full term parturition. Bacteria can also be found in the blood, urine, milk and semen; shedding in milk and semen can be prolonged or lifelong (Aqasthya *et al*., 2007). Infection occurs by ingestion and through mucous membranes and possibly intact skin. The mammary gland can be infected by direct contact; in cattle, the udder can be colonized by *B. abortus*, *B. melitensis* or *B. suis* on the hands in farm workers *B. suis*, *B. ovis* and *B. canis* can be spread venereally; venereal transmission of *B. abortus* can

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occur but is rare. Some *Brucella* species can be transmitted vertically (Corbel, 1997; Aqasthya *et al*., 2007). Several epidemiological factors such as age, sex, breed, lactation number, herd size and living condition influence the seroprevalence of brucellosis (Ghain *et al*., 1998; Aqasthya *et al*., 2007).

Man is infected by animal's brucellosis through direct or indirectly by ingestion of animal products as well as by inhalation of air borne agents. The animals that are commonly known to serve as source of human infection are goat, sheep, cattle and swine, dogs have long been known as carriers of *Brucellae* (Baldwin and Goenka, 2006). Brucellosis is usually an occupational disease; most cases occur in abattoir workers, veterinarians, hunters, farmers and livestock producers. Sometimes infection occurs after drinking raw milk or eating unpasteurized cheese (Celebi *et al*., 2007). Man is susceptible to infection by *Br. melitensis* (Malta fever), the susceptibility for this species is very high and can cause epidemic infection almost cause manifest illness, conversely the pathogenicity of the *Br. abortus* (Bang's disease) is low (Basrai *et al*., 2007). The same authors added that *Br. melitensis* is the most pathogenic and invasive species for man, followed by *Br. suis*, *Br. abortus* and *Br canis*.

Brucellosis is often diagnosed by serology. In cattle, agglutination tests are used to detect antibodies in serum, milk, whey and semen. The most commonly used tests are the Buffered *Brucella* Antigen Tests (BBAT), also known as the card and plate agglutination tests. Tube agglutination tests may also be used. An Enzyme-linked Immunosorbent Assay (ELISA) is available for milk or serum. The milk ring test can be used to screen bulk milk samples for *B. abortus*. Other, less commonly used, serologic tests include complement fixation, rivanol precipitation and acidified antigen procedures, fluorescence polarization tests are being developed (Uzal *et al*., 1996; McGiven *et al*., 2006).

The objective of this study was to investigate the seroprevalence of brucellosis among cattle and cattle-owners at selected area of Khartoum state.

MATERIALS AND METHODS

Sources and Type of Samples

One hundred seventy five sera from unvaccinated cow were collected for serological examination using (standard tube agglutination test) for detection of presence of *Br. melitensis* and *Br. Abortus* antibodies. Fifty milk samples were examined for detection of *Br. abortus* and 25 cow-milkers sera were examined serologically at Khartoum state. This study was done at laboratory of Microbiology, Faculty of Science and Technology, El-Neelain University, at 2006.

Collection of Samples

Serum Samples

Blood was collected from cow by venpuncture of jugular veins using vactainer tubes with needle holders, cow-milkers (human) samples were collected from vein by using sterile syringe. All blood samples were transferred into blood containers then centrifuged at 3000 RPM for 3 min to obtain sera.

Milk Samples

The teat were disinfected with alcohol and then allowed to dry. The first streak of milk was discharged then milk was siphoned into sterile tubes.

Standard Tube Agglutination Test

This was done according to Macmillan (1990), six tubes containing 1 mL of physiological saline (diluents) were prepared, to the first tube 1000 μL from sera samples were added, mixed gently and then 1000 μL transferred to second tube, these was repeated in all tubes to make serial dilution, one drop of *Br. melitensis* antigen was added into each tube and incubated for one to two hours at 37°C. The same method was applied using *Br. abortus* antigen in other six tubes. The result was compared with known positive and negative control.

Milk Ring Test (MRT)

These was done according to Morgan *et al*. (1978), 0.03 mL of *Brucella abortus* antigen was added to 1000 μL of milk, mixed well and incubated at 37°C for one hour and then examined for ring formation.

RESULTS

From 175 unvaccinated cattle, 84 (48%) were showed positive serum agglutination reaction towards *Br. melitensis* antigen. while *Br. abortus* were identified in 44 (26.9%). The highest incidence of *Br. melitensis* positive sera was shown in age group of more than 10 years old as 46 (68.7%) followed by age group of 5-10 years old 32 (42.1%) and the lowest incidence was shown in cattle with age group less than 5 years old as 6 (18.8%). *Br. abortus* was showed highest incidence in age group of more than 10 years old 32 (47.8%) followed by age group of 5-10 years old which shown 15 (14.7%) positive while the age group of less than 5 years old showed negative result toward *Br. abortus* (Table 1). Mix infection was also detected, the highest mix infection was observed in the age group of more than 10 years old 26 (38.8%) followed by age group of 5-10 years old which shown 12 (15.8%) while in age group of less than 5 years old showed negative presence of mix infection (Table 2).

The highest incidence of *Br. melitensis* was shown in cows lactating between 6-10 times as 22 (12.6%) followed by cows at first lactation, 20 (11.4%) then 14 (8%) shown in cows at the second milking times, these followed by 9 (5.1%) in cows at 4 times lactation period (Table 3). The lowest were shown in cows at the third lactating times and more than 10 lactating times. On the other hand *Br. abortus* was showed highest incidence in cows that lactating between 6 and 10 times the lowest 2 (7.4%) was observed in cows that lactating more than 10 times others shown range between 7 (4%) and 4 (2.3%).

Table 1: Number and percentage of *Br. melitensis* and *Br. abortus* in cattle according to age

		Br. melitensis		*Br. abortus*	
Age group	Total No.	Positive (%)	Negative (%)	Positive (%)	Negative (%)
Less than 5 years	32	6 (18.8)	26 (81.2)	0 (0)	32 (100)
5-10 years old	76	32 (42.1)	44 (57.9)	15 (19.7)	61 (80.3)
More than 10 years old	67	46 (68.7)	21 (31.3)	32 (47.8)	35 (52.2)
Total	175	84 (48)	91 (52)	47 (26.9)	128 (73.1)

Table 2: Incidence of mix infection in cattle according to age

Age group	No. of examined animal	No. of mixed infection	Percentage
Less than 5 years old	32	0	0.0
5-10 years old	76	12	15.8
More than 10 years old	67	26	38.8
Total	175	38	21.7

Table 3: Number and percentage of Brucellosis according to lactation No.

Lactation No.	Total examined No.	*Br. melitensis* positive (%)	*Br.abortus* positive (%)	Negative (%) for *Br.abortus* and *Br. melitensis*
1	64	20 (11.4)	4 (2.3)	43 (24.6)
2	31	14 (8)	7 (4)	16 (9.1)
3	13	6 (3.4)	6 (3.4)	7 (4)
4	12	9 (5.1)	7 (4)	3 (1.7)
5	12	7 (4)	5 (2.9)	6 (3.4)
6-10	37	22 (12.6)	13 (7.4)	11 (6.3)
>10	6	6 (3.4)	2 (1.1)	-
Total	175	84 (48)	44 (26.9)	86 (49.1)

- : Not detected

Table 4: Number and percentage of brucellosis according to titre

Brucella spp.	No. of positive	No. (%) of 1/160	No. (%) of 1/320
Brucella melitensis	84	58 (33.1)	26 (14.9)
Brucella abortus	44	43 (24.6)	1 (0.6)

From 84 positive samples of *Br melitensis* 58 (33.1%) gave titre of 160, 26 (14.9%) gave titre 320 while in those 44 positive *Br. abortus*, 43 (24.6%) showed titre of 160 and only 1 (0.6%) showed titre 320 (Table 4).

Out of 25 milkers sera 10 (40%) were showed positive result with *Br. melitensis* with titre of 160, while 15 (60%) showed negative result.

From 50 milk samples, 40 (80%) showed positive reaction witk Milk Ring Test (cream ring colored) toward *Br. abortus* and ten (20%) showed negative reaction (pink suspension colored).

DISCUSSION

In this study the frequency of detection of brucellosis was very high. The high incidence of brucellosis was detected in cattle of more than 10 years old. These might be due to impaired in immune system due to ageing and diseases occurrence, the climate of the Sudan also may facilitate the stress of immune system and these also might explain the presence of mix infection. The bad hygienic measurements increase the hazardous risk and facilitate the spread of the infection.

The high incidence of the brucellosis at cows lactating for first time (11.4%) is unexpected and of serious economical values. *Br. melitensis* is detected in all age grouping, while *Br. abortus* not detected in those with less than 5 years old these might be due to anatomical structure and physiological activities of cattle at less than five years old and that *Br. abortus* associated with abortion and infectivity appeared in the old animals, the detection of the presence of positive reactors among examined cattle confirm the previous findings of El-Sharif (1994); El-Ansary and Mohammed (2001) who reported the presence of brucellosis in rural areas of The Sudan, present detection of positive brucellosis in the selected region is of serious indication because this selected region is part of the capital city of the Sudan that characterized by heavy milk production which directly distributed or marketing as raw milk to all other parts of the capital city. The presence of brucellosis in this area might explain by the movement and marketing activities.

In present results the occurrence of positive reactive human sera with titre of 160 is considered significance and diagnostic for human infection, these might confirm the findings of Musa (1995), El-Amin *et al*. (2001), Garin and Bastuuji (2001), Alton and Forsyth (2002), Baldwin and Goenka (2006) and Celebi *et al*. (2007) who isolated *Br. melitensis* from human cases. In our results all positive human sera observed with *Br. melitensis*, this supported the finding of Basrai *et al*. (2007) who reported that human is more susceptible to *Br. melitensis* than *Br. Abortus*. The infection might be due to direct exposed with animal or consumption of raw milk or milk by-products.

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