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Seroprevalence and Risk Factors of Brucellosis in Sheep in North Kordofan State Sudan

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Abstract: *A cross-sectional study was carried out from April to July/2012 to estimate the prevalence of brucellosis in sheep and to investigate potential risk factors associated with the disease in North Kordofan state. A total of 318 serum samples were collected from sheep. 2.5% (8/318; 95% CI from 0.78 to 4.22) and 22.0% (70/318; 95% CI from 17.5 to 26.6) prevalences were parallel estimated by Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (STA). All the RBPT and SAT positive serum samples were serially tested by a Competitive Enzyme Linked Immunosorbent Assay (ELISA). The investigated potential individual risk factors included: localities, breeds, age groups, body condition, sexes and parity while herd size, mixed herd, housing, feeding and drinking equipments, having aborter animals in the herd, disposal of the foetal membranes after abortion, practicing milking hygiene and presence of insects and dogs in the herd were the management risk factors. Univariate analysis using chi- square, with a confidence interval of 95% at a p-value of ≤0.05 was employed to identify potential risk factors associated with RBPT- and SAT-positivity statuses for brucellosis infection in sheep. With exception of age (Chi^2 = 5.69, p-value = 0.017) in SAT-positive status, none of the other individual or management risk factors had an effect on the occurrence of brucellosis in sheep in North Kordofan State, neither for RBPT- nor for SAT-seropositive statuses. Multivariate analysis using logistic regression was not used because all risk factors had no effect on the spreading of the disease in the univariate analysis using chi square at p-value of ≤0.05. It is concluded that brucellosis in sheep is probably not a significant public health problem in North Kordofan State. However, more studies investigating potential risk factors that could enhance the spread and transmission of brucellosis in sheep in the Sudan are recommended, besides, eradication plans should take place when still the disease is at this low prevalence as the needed effort and cost to manage the disease would be small.*

Keywords: *Seroprevalence, risk factor, brucellosis, sheep, Sudan*

I. Introduction

Brucellosis is one of the most important bacterial zoonoses with a cosmopolitan distribution [24, 27, 32, 36]. It is an infectious disease, almost invariably transmitted by direct or indirect contact with infected animals or their products [24]. The disease is caused by gram-negative coccobacillae bacteria which belong to the genus Brucella which includes *Brucella melitensis* and *B. ovis* as well as many other species [32]. The natural reservoirs of the species B. melitensis are basically goats and sheep but also cattle and swine. However, *B. ovis* is primarily afflicting sheep [32].

The significance of the disease is due to its zoonotic and economic impacts [7, 32]. It can be transmitted to people in contact with infected animals or consuming their products [32]. However, the causative agent has a very low infectious dose; only 10 organisms of *B. melitensis* are sufficient to cause an infection in man [32]. Furthermore, in animals, brucellosis causes severe economic losses as result of stormy abortions or reproductive failure, sterility and reduced milk production rates, besides to that, it adds to the burden shouldered by the farmers; the costs of control and management. Also brucellosis of animals reduces the Foreign Exchange Earnings (FEE) by denying exportation of sheep to international markets [7, 36].

Pre-requisition of good knowledge on risk factors associated with the occurrence of infectious such as brucellosis in sheep is imperative for the correct design and effective and efficient implementation of disease control strategies too [33]. Nonetheless, important factors that contribute to the spread of brucellosis in sheep include: farming system and practices, farm sanitation, livestock movement, mixing and trading of animals, and sharing of grazing grounds and watering points [13, 14, 19]. Further complications arise through wild animal reservoirs which may also carry and transmit the disease [10, 11]. Abortion materials characteristically contain high numbers of brucellae and consequently pose significant infection risks if not properly handled and disposed off. Similarly, environmental contamination contributes to additional spread among animals [32]. Infected non-

pregnant livestock may not demonstrate clinical signs of infection which makes the control and prevention more challenging [32]. The disease appears in two distinguishable entities in sheep; classic brucellosis and ram epididymitis [2]. In livestock, Brucella results in stormy abortions, reduced fertility and weak offspring. In addition, symptoms such as hygromas in cattle or orchitis and spondylitis can also be seen [8].

Several studies have been conducted in the in the Sudan to investigate brucellosis in animals such as sheep, goat, camels and cattle [20]. Most of these studies focused on the different aspects of the disease but not risk factors and epidemiology. The objectives of this study were to estimate the prevalence of brucellosis in sheep in North Kordofan state and to investigate the risk factors associated with it.

II. Materials And Methods

2.1. Study area

North Kordofan lies in the arid and semi-arid zones between latitude 11.15-16.45º N and longitude 27-32.15º E. It borders the Northern state in the north, Northern and Southern Darfur states in the west, West and South Kordofan states in the south, and the White Nile and Khartoum states in the east. Soil types are about 55% sand or gouze, 20% gerdud, 15% alluvial land and 10% clay land. Annual rainfall is concentrated in a single relatively short summer season during June to September and the region enjoys an annual rainfall of 0 to 500 mm. Kordofan falls in the grass-land and wood-land savannahs; it has abundant fodder and grazing areas during rainy seasons during which animals are trekked by pastoralists to the northern part of the region while during dry seasons animals are trekked to the southern part of the region up to the Bahar Al-Gazal River in South Sudan. Agriculture and livestock comprise about 70% of the economic activity in Kordofan [33, 45]. A mixture of farming systems are practiced in the region including nomadic, sedentary and semi-sedentary animal production systems. Kabashi and Hamarri desert sheep, the main breeds raised in the region, are considered the best breeds for live sheep and the second-best breed for meat. The bulk of the Sudan's live sheep exports and meat for local consumption are from this region. In addition, most of the large sheep (average 35–45 kg live weight) and high-quality lambs purchased during the annual Hajj and Ramadan religious festivals originate from Kordofan region [42]. The region has an estimated livestock population of 24.665.761 animals of which 10.131.693 are sheep [41].

2.2. Study design and sampling strategy

A cross-sectional study design, from April to July/2012, employing a multistage sampling method was used in the current survey as described by Martin et al. [16] and Thrusfield [25]. Out of the 13 localities of North Kordofan state, 5 were conveniently selected, namely: Shaikan, Alrahad, Baarah, Ummruwaba and Elkhoway. Within the selected localities smaller administrative units and/or villages were further conveniently selected. Umsimaimah and Abu-Haraz in Shaikan locality; Elkemtan, Fangoga and Elrahmania in Alrahad locality; Shrim Elnazir and Shrim Elsheikh in Baarah locality; Um Burma, Kargni, Elswima and Elabedab in Ummruwaba locality and Nasharbo and Bani badur in Elkhway locality. In each selected administrative unit and/or village sheep flocks and individual animals were randomly or conveniently sampled [25].

2.3. Sample size

The sample size (n) for determining the prevalence of brucellosis in sheep in North Kordofan was calculated according to Thrusfield [25] and based on the following parameters: 95.0% level of confidence, ±5% desired level of precision and the expected prevalence of brucellosis in sheep of 12.2% [12]. By using the following formula:

$$n = \frac{(1.96)^2 P_{exp.} (1 - P_{exp.})}{d^2}$$

Where:

n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

The required sample size was found to be 159 animals. This number was inflated 2-fold to account for the effect of randomness and representativeness in multistage sampling strategy with more than two levels [25]. Thus, total n was 318 serum samples from North Kordofan.

2.4. Collection of samples

About 3 to 5 ml of blood was collected aseptically from the jugular vein of animals in a plain tube with serum clot activator. The plain tubes were kept in an upright position at 25 ºC for about 2 hours. The separated serum was collected in a screw capped plastic vials and transported to the laboratory of the Veterinary Research Institute (VRI), El-Obeid where they were stored at -20ºC till used.

2.5. Rose Bengal Plate Test (RBPT)

RBPT was carried out as described by Ferede et al. [9]. The interpretation of the result is done according to the degree of agglutination. Agglutination is recorded as 0, +, ++ and +++. A score of 0 indicates the absence of agglutination; a score of + indicate barely visible agglutination; ++ indicates fine agglutination and +++ indicates coarse clumping. Those samples with no agglutination (0) were recorded as negative while other were recorded as positive. The test was carried out at the facilities of the College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum North, the Sudan.

2.6. Serum Agglutination Test (SAT)

SAT was carried out as described by OIE [18]. The degree of Brucella agglutination in a serum was expressed in International Unit (IU) per ml. A serum containing 40 or more IU per ml was considered to be positive. The test was carried out at the facilities of the Veterinary Research Institute (VRI), Soba, Khartoum, the Sudan.

2.7. Competitive Enzyme Linked Immunosorbent Assay (cELISA)

The cELISA kit was obtained from the Veterinary Laboratory Agency, Surrey, United Kingdom. Reagents were reconstituted as directed by the manufacturer. These included diluting buffer, washing solution, stopping solution, conjugate and control sera. The test procedure was carried out as per the manufacturer's protocol. The lack of color development indicated that the sample tested was positive. A positive / negative cut-off was calculated as 60% of the mean of Optical Density (OD) of the 4 conjugate control wells. Any test sample gave an OD equal or below this value was regarded as being positive. The test was carried out at the facilities of the Veterinary Research Institute (VRI), Soba, Khartoum, the Sudan.

2.8. Questionnaire survey

A questionnaire for each flock was completed by the herder and/or owner through an interview to identify possible independent variables (potential risk factors) associated with the presence of sero-positive sheep in the flock [15]. Potential individual risk factors and their categories were as follow: sex (male and female), age (≤1 and >1 years), breed (Hamarri and Kabashi), body condition (good and poor), and parity (0, 1-3 and >3). However, management risk factors included: herd size (≤ 50, 51 – 100 and > 100 animals), housing (indoor and outdoor), disposal of fetal membranes (yes and no), feeding and drinking equipments (present and absent), milking hygiene or washing the udder before and after milking (yes and no), mix with other animal species (yes and no), previous illness (yes and no), presence of dogs (yes and no) and presence of insects (yes and no) and history of abortion (yes , no).

2.9. Data Analysis

The Statistical Package for Social Sciences (SPSS) for Windows® version 18.0 (SPSS Inc., Chicago, Illinois) was used for all appropriate statistical analyses. Descriptive statistics of the variables were obtained. For each variable (age, sex, breed, body condition, parity and locations), frequencies (number of observations within variable) and prevalence rates by cross-tabbing (number of positive valid samples/number of individuals sampled in the variable) were obtained. Hypotheses of differences of age group, breed, sex, and locations between test-positive and test-negative animals were first tested by univariate analysis by means of the 2-tailed chi-square test. In a second step, a logistic regression model was used to assess the association between the potential individual and management risk factors and the outcome variable brucellosis serological status. Associations in the Chi-square test and the logistic regression model were deemed significant when $p \leq 0.05$ [33].

III. Result

3.1. The overall seroprevalences of brucellosis in sheep

A total of 318 serum samples were collected from sheep in North Kordofan state to estimate the seroprevalence of brucellosis by using RBPT and SAT in parallel. Furthermore, all the samples which tested RBPT- and/or SAT-positive were subjected to serial testing by cELISA. The overall seroprevalences were 2.5% (n = 8) with 95% CI from 0.78 to 4.22 and 22.0% (n = 70) with 95% CI from 17.5 to 26.6, by RBPT and SAT, respectively (Table 1 and 3). However, no any; 0.0% (0/8) with 95% CI from 0.00 to 0.00, of the samples which were RBPT-positive was positive by cELISA while only 1.4% (1/70) with 95% CI from -1.35 to 4.15 of the samples tested SAT-positive were positive by the cELISA.

3.2. RBPT-estimated seroprevalences and risk factors

There were no statistical significant differences at p-value ≤0.05 in the seroprevalences estimated by using RBPT for the variables of the individual risk factors (localities, breeds, age groups, body condition, sexes and parity). As presented in Table 1, Shaikan locality (6.3%, 4/64, with 95% CI from 0.35 to 12.25), Hamarri

breed (2.9%, 7/244, with 95% CI from 0.79 to 5.01), age group >1 years (2.7%, 8/297, with 95% CI from 0.86 to 4.54), good body condition (2.6%, 8/311, with 95% CI from 0.83 to 4.37), female (2.9%, 7/244, with 95% CI from 0.79 to 5.01) and animals that gave birth > 3 times (7.7%, 4/52, with 95% CI from 0.45 to 15.0), were showing higher prevalences than the other 4 localities, Kabashi breed, age group ≤1 years, poor body condition, males and animals that gave birth 0 and 1 – 3 times, correspondingly.

The proportions of the sero-positive differ between localities, breeds, age groups, body condition, sexes and parity. In the univariate analysis using chi square, locality (Chi^2 = 5.820, p-value = 0.213), breed (Chi^2 = 0.533, p-value = 0.465), age (Chi^2 = 0.580, p-value = 0.446), body condition (Chi^2 = 0.185, p-value = 0.667), sex (Chi^2 = 0.533, p-value = 0.465) and parity (Chi^2 = 6.79, p-value = 0.330) were not significantly associated with RBPT positive status for brucellosis infection in sheep (Table 1).

The number of brucellosis-seropositives by using RBPT were investigated between herd sizes (≤ 50, 51 – 100 and > 100), mixed herds (yes or no), housing (indoor or outdoor), feeding and drinking equipments (present or absent), having aborter animals in the herd (yes or no), disposal of the fetal membranes after abortion (yes or no), practicing milking hygiene (wash the udder before and after milking or not) and presence of insects and dogs (yes or not). However, in the univariate analysis, using chi square with a p-value of ≤0.05, herd sizes (Chi^2 = 4.565, p-value = 0.102), mixed herds (Chi^2 = 0.551, p-value = 0.458), housing (Chi^2 = 2.937, p-value = 0.087), feeding and drinking equipments (Chi^2 = 1.604, p-value = 0.205), having aborter animals in the herd (Chi^2 = 0.370, p-value = 0.543), disposal of the fetal membranes after abortion (Chi^2 = 1.991, p-value = 0.158), practicing milking hygiene (Chi^2 = 0.131, p-value = 0.717), presence of insects (Chi^2 = 0.719, p-value = 0.396) and presence of dogs in the herds (Chi^2 = 0.378, p-value = 0.539) were not significantly associated with RBPT-sero-positivity (Table 2).

The logistic regression model was not used to assess the association between the RBPT-sero-positivity and the individual and the management risk factors combined together as they were all not significantly associated with RBPT positive status for brucellosis infection in sheep in the univariate analysis using chi square.

3.3. SAT-estimated seroprevalences and risk factors

The statistical differences at p-value ≤0.05 between the seroprevalences estimated by using SAT for the variables of the individual risk factors were not significant (Table 3). However, among the 5 surveyed localities, Ummruwaba locality showed the highest seroprevalence (30.2%, 19/63, with 95% CI from 18.9 to 41.5) and sheep belonging to Kabashi breed had a seropositivity of 23.0% (17/74) with 95% CI from 18.9 to 41.5 which was higher than the seropositivity reported among the sheep belonging to Hamrri breed. The age group ≤1 years (42.9%, 9/21, with 95% CI from 21.7 to 64.1) had a higher SAT-sero-positives than >1 years age group. Additionally, poor body condition (42.9%, 3/7, with 95% CI from 6.24 to 79.6), females (23.4%, 57/244, with 95% CI from 18.1 to 28.7) and animals which gave birth 1 - 3 times (22.6%, 30/133, with 95% CI from 18.0 to 27.3), were showing higher prevalences than the animals with good body condition, males and animals which gave birth 0 and > 3 times.

The ratios of the sero-positive sheep varied amongst the variables of the studied individual risk factors. When the chi square test was used to establish the relationship of each factor alone to the occurrence of the disease, only age (Chi^2 = 5.69, p-value = 0.017) was found to be significantly associated with SAT-positive status for brucellosis infection in sheep (Table 3). However, the same statistical analysis revealed that none of the following factors: locality (Chi^2 = 6.338, p-value = 0.175), breed (Chi^2 = 0.52, p-value = 0.820), body condition (Chi^2 = 1.812, p-value = 0.178), sex (Chi^2 = 1.11, p-value = 0.292) and parity (Chi^2 = 0.05, p-value = 0.976) was significantly associated (p≤0.05) with the SAT-positivity for brucellosis infection in sheep (Table 3).

The univariate analysis, using chi square with a p-value of ≤0.05, showed that ≤ 50, 51 – 100 and > 100 herd sizes (Chi^2 = 2.334, p-value = 0.311), whether herds were mixed or not (Chi^2 = 2.09, p-value = 0.148), indoor or outdoor housing (Chi^2 = 2.21, p-value = 0.136), presence or absence of feeding and drinking equipments (Chi^2 = 0.87, p-value = 0.136), having aborter animals in the herd or not (Chi^2 = 0.395, p-value = 0.530), proper and prompt disposal of the fetal membranes or not (Chi^2 = 1.078, p-value = 0.299), practicing milking hygiene or not (Chi^2 = 1.434, p-value = 0.231), presence of insects or not (Chi^2 = 3.010, p-value = 0.083) and presence of dogs in the herds or not (Chi^2 = 1.602, p-value = 0. 206) were not significantly associated with SAT-seropositivity (Table 4).

With exception of age, none of the other individual or management risk factors had a p-value of ≤0.05 when the chi square test was performed with SAT-seropositivity. Thus the combined effect of these factors all together cannot be established using logistic regression.

IV. Discussion

Generally, the seroprevalence of brucellosis differs from one country to another, among different flocks or herds, different animal species and across geographical locations and animal production systems [21, 22, 29,

30]. However, antibodies against brucellosis have been reported previously in animal populations and human beings in the Sudan [20, 36, 39]. In this study, the seroprevalence estimated by RBPT (2.5%; 8/318 with 95% CI from 0.78 to 4.22) agreed with that of Yesuf et al. [26] in Ethiopia and Rahman et al. [22] in Bangladesh in goats. Both Yesuf et al. [26] and Rahman et al. [22] reported a sero-prevalence of 2.5%. Conversely, it was higher than of Ferede et al. [9] in Ethiopian sheep and goats (1.2%; 6/500), Cadmus et al. [6] in sheep in Nigeria (0.0%; 0/54) and Rahman et al. [22] in sheep in Bangladesh (1.3%). In addition, to the reports of Omer et al. [20] who found the following prevalences in sheep in the Sudan: 0.10%, 0.4% and 2.1% in a period of three years from 2004 to 2006. On the other hand, the RBPT-seropositive percentage reported in this study was lower than that reported in Egypt of 26.7%, 12.2% and 29.3% by Kaoud et al. [15], Hegazy et al. [12] and Abdel Hafez et al. [1], in Iran of 4.2% by Akbarmehr and Ghiyamirad [3], in Nigeria of 14.5% by Bertu et al. [5], in Ethiopia of 5.6%, 3.2% and 3.6% by Teshale et al. [24], Ashenafi et al. [4] and Nigatu et al. [35] and in Bangladesh of 3.08% and 9.4% by Rahman et al. [21] and Rahman et al. [23]. Furthermore, the overall SAT-seroprevalence of antibodies against brucellosis in sheep in North Kordofan state was found to be 22.0% (70/318 with 95% CI from 17.5 to 26.6). This finding was higher than that of Bertu et al. [5] in Nigeria (5.2%) and Mustafa et al. [17] in Lahore (0.0%). While inversely, it was lower than that reported by Abdel Hafez et al. [1] in Egypt (27.0%) in sheep and goats and by Hamidullah et al. [43] in Pakistan (32.5%). These observed variations in the RBPT- and SAT-seropositivity percentages could be explained by the differences in the tested sample sizes (n) in each study, differences in the animal production systems and husbandry and differences of the geographical regions and ecological settings.

Merely few studies addressed risk factors associated with brucellosis sero-positivity in sheep in the Sudan. In the current study, the univariate analysis using chi square revealed that none of the individual risk factors was significantly associated with RBPT-positive status; locality (Chi^2 = 5.820, p-value = 0.213), breed (Chi^2 = 0.533, p-value = 0.465), age (Chi^2 = 0.580, p-value = 0.446), body condition (Chi^2 = 0.185, p-value = 0.667), sex (Chi^2 = 0.533, p-value = 0.465) and parity (Chi^2 = 6.79, p-value = 0.330). For SAT, only age (Chi^2 = 5.69, p-value = 0.017) was found to be significantly associated with positive status. Other factors including: locality (Chi^2 = 6.338, p-value = 0.175), breed (Chi^2 = 0.52, p-value = 0.820), body condition (Chi^2 = 1.812, p-value = 0.178), sex (Chi^2 = 1.11, p-value = 0.292) and parity (Chi^2 = 0.05, p-value = 0.976) were not significantly ($p \leq 0.05$) associated with the SAT-positivity. Some of these findings were dissimilar to the findings of Mikolon et al. [28] who found out that brucellosis-positivity was significantly associated with location, breed and sex in small ruminants. Mikolon et al. [28] observed raising of animals in specific location in Mexico and the presence of females of the `La Mancha' breed in the herd increase the percentage positivity of brucellosis. But regarding origin of the animals our finding was typifying the observation of Nigatu et al. [35] who concluded that origin of the animals, whether were from high or low lands in Ethiopia, had not significant effect on brucella-seropositive. Furthermore, results of the present study showed that age had no significant effect on the status of being RBPT-positive, confirming the report of Mikolon et al. [28] who observed that having five years or less in goat flocks was not significant at p-value ≥ 0.10. But the result of SAT-positive status regarding the effect of age was totally reverse. Once more, consistent with the outcome of the chi square test regarding the relationship between age and being RBPT-serpositive and inconsistent with SAT-serpositivity in this study, Agab [39] and Wadood et al. [37] concluded that age was potentially not a significant risk factor that could probably influence the epidemiology of brucellosis in animals, although they did observe significant statistical differences in seroprevalences among various age groups and the antibody titer against brucellosis appeared to be lower in younger animals than in adults. Contrary to the findings of this study, Nigatu et al. [35] noted that brucellosis prevalence varied significantly ($p \leq 0.05$) with body weights where higher prevalence was observed in poor body conditioned than that of medium and good body conditions. But our finding agreed with Ahmed and Munir [40] and Wadood et al. [37] who reported that the prevalence difference among the animals belonging to different groups according to their body condition score was none significant. Moreover, results of this study confirmed the finding of Muma et al. [38] and Wadood et al. [37] that the prevalences among males and females were statistically none significant different and the disease was not related to sex. In this study parity the observed difference in the seroprevalence was statistically non significant. Similar observations were made by Berhe et al. [44] and Wadood et al. [37].

Nevertheless, when the relationship, using chi square with a p-value of ≤ 0.05, between RBPT- and SAT-brucellosis-seropositives and the management risk factors including: herd sizes (≤ 50, 51 – 100 and > 100 animals), mixed herds (yes or no), housing (indoor or outdoor), feeding and drinking equipments (present or absent), having aborter animals in the herd (yes or no), disposal of the fetal membranes after abortion (yes or no), practicing milking hygiene (wash the udder before and after milking or not) and presence of insects and dogs in the herds (yes or not), was established, all factors came out not significantly related to RBPT- or SAT-sero-positivity. Disagreeing with our findings Mikolon et al. [28] identified larger herd sizes, mixed herds i.e. presence of sheep and other ruminants, and presence of dogs in the herd as significant ($p<0.05$) risk factors for goat-herds seropositivity for brucellosis. Ghani et al. [31] and Akhter et al. [29] stated that numerous risk

factors, such as: age, sex, breed, lactation number, herd size and living conditions might determine the seroprevalence of brucellosis in animals. However, agreeing with our findings Akhter et al. [29] found none of these risk factors was significantly ($p \geq 0.05$) associated with brucellosis in sheep.

Although the differences reported between the seroprevalnces of the variables of one risk factor were not statistically significant, results of this study did confirm the observations of Rahman et al. [21], Lone et al. [34], Islam et al. [30], Agab [39], Wadood et al. [37] and Akhter et al. [29] that diverse percentage positivities could occur due to variation in the environment, sex, age, breed and other factors.

V. Conclusions And Recommendations

It can be concluded that sheep brucellosis according to serological diagnosis is prevailing in North Kordofan State at a low rate. Therefore, brucellosis in sheep is potentially not a significant public health problem. Additionally, with exception of age in SAT-positive status, none of the other individual or management risk factors had an effect on the occurrence of brucellosis in sheep in North Kordofan State, neither for RBPT- nor for SAT-seropositive status. However, more studies investigating potential risk factors that could enhance the spread and transmission of brucellosis in sheep in the Sudan are warranted. Also controlling, managing and eradicating the disease at this low prevalence should be a priority as it will take only a small effort and cost.

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Table 1: Estimated Seroprevalences of brucellosis in sheep by locality, breed, age, body condition, sex and parity in North Kordofan State by RBPT and Univeriate Analysis for the Association between brucellosis in sheep and individual animal risk factor using the Chi square test (from April to July/2012)

Risk Factors	No. of tested samples	No. of positive samples	Sero-prevalence (%)	95% CI Lower - Upper	χ^2	p-value
Localities					5.820	0.213
Elkhoway	64	2	3.1[a]	-1.15 - 7.35		
Shaikan	64	4	6.3[a]	0.35 - 12.25		
Baarah	63	0	0.0[a]	0.00 - 00.00		
Alrahad	64	1	1.6[a]	-1.47 - 4.67		
Ummruwaba	63	1	1.9[a]	-1.47 - 5.27		
Breeds					0.533	0.465
Kabashi	74	1	1.4[a]	-1.28 - 4.08		
Hamarri	244	7	2.9[a]	0.79 - 5.01		
Age groups (Yrs)					0.580	0.446
≤1	21	0	0.0[a]	0.00 - 0.00		
>1	297	8	2.7[a]	0.86 - 4.54		
Body condition					0.185	0.667
Poor	7	0	0.0[a]	00.0 - 0.00		
Good	311	8	2.6[a]	0.83 - 4.37		
Sex					0.533	0.465
Male	74	1	1.4[a]	-1.28 - 4.08		
Female	244	7	2.9[a]	0.79 - 5.01		
Parity					6.79	0.330
0	133	2	1.5[a]	-0.57 - 3.57		
1 - 3	133	2	1.5[a]	-0.57 - 3.57		
> 3	52	4	7.7[a]	0.45 - 15.0		
Total	**318**	**8**	**2.5**	**0.78 - 4.22**		

different superscripts indicate significant difference at $p \leq 0.05$

Table 2: Results of univariate associations of herd size, mixed herds, housing, presence of equipment, abortion, disposal of foetal membranes, presence of insects and dogs in herds with RBPT brucellosis-sero-positivity in sheep in North Kordofan State using the Chi square test (from April to July/2012).

Risk factors	Number of samples	Number of positives	% positives	χ^2	p-value
Herd size				4.565	0.102
≤ 50	26	0	0.0		
51 - 100	66	4	6.1		
> 100	226	4	1.8		
Mixed herd				0.551	0.458
Yes	20	0	0.0		
No	298	8	2.7		
Housing				2.937	0.087
Indoor	50	3	6.0		
Outdoor	268	5	1.9		
Equipments				1.604	0.205
Present	266	8	3.0		
Absent	52	0	0.0		
Abortion				0.370	0.543
Yes	153	5	3.3		
No	165	3	1.8		
Disposal of FMs				1.991	0.158
Yes	203	7	3.4		
No	115	1	0.9		
Milking hygiene				0.131	0.717
Washed	5	0	0.0		
Unwashed	313	8	2.6		
Insects				0.719	0.396
Yes	300	7	2.3		
No	18	1	5.6		
Dogs				0.378	0.539
Yes	14	0	0.0		
No	304	8	2.6		

Table 3: Estimated Seroprevalences of brucellosis in sheep by locality, breed, age, body condition, sex and parity in North Kordofan State by SAT and Univeriate Analysis for the Association between brucellosis in sheep and individual animal risk factor using the Chi square test (from April to July/2012)

Risk Factors	No. of tested samples	No. of positive samples	Sero-prevalence (%)	95% CI Lower - Upper	χ^2	p-value
Localities					6.338	0.175
Elkhoway	64	8	12.5[b]	04.4 - 20.6		
Shaikan	64	13	20.3[b]	10.5 - 30.2		
Baarah	63	16	25.3[b]	14.6 - 36.0		
Alrahad	64	14	21.9[b]	11.8 - 32.0		
Ummruwaba	63	19	30.2[b]	18.9 - 41.5		
Breeds					0.52	0.820
Kabashi	74	17	23.0[b]	18.9 - 41.5		
Hamarri	244	53	21.7[b]	16.5 - 26.9		
Age groups (Yrs)					5.69	0.017
≤1	21	9	42.9[b]	21.7 - 64.1		
>1	297	61	20.5[b]	15.9 - 25.1		
Body condition					1.812	0.178
Poor	7	3	42.9[b]	6.24 - 79.6		
Good	311	67	21.5[b]	16.9 - 26.1		
Sex	74	13	17.6[b]	8.90 - 26.3	1.11	0.292
Male	244	57	23.4[b]	18.1 - 28.7		
Female						
Parity					0.05	0.976
0	133	29	21.8[b]	17.2 - 26.4		
1 - 3	133	30	22.6[b]	18.0 - 27.3		
> 3	52	11	21.2[b]	10.1 - 32.3		
Total	**318**	**70**	**22.0**	**17.5 - 26.6**		

different superscripts indicate significant difference at p≤0.05

Table 4: Results of univariate associations of herd size, mixed herds, housing, presence of equipment, abortion, disposal of foetal membranes, presence of insects and dogs in herds with SAT brucellosis-sero-positivity in sheep in North Kordofan State using the Chi square test (from April to July/2012).

Risk factors	Number of samples	Number of positives	% positives	χ^2	p-value
Herd size				2.334	0.311
≤ 50	26	3	11.5		
51 - 100	66	13	19.7		
> 100	226	54	23.9		
Mixed herd				2.09	0.148
Yes	20	7	35.0		
No	298	63	21.1		
Housing				2.21	0.136
Indoor	50	7	14.0		
Outdoor	268	63	23.5		
Equipment				0.87	0.136
Present	266	56	21.1		
Absent	52	14	27.0		
Abortion				0.395	0.530
Yes	153	34	22.2		
No	165	36	21.8		
Disposal of FMs				1.078	0.299
Yes	203	41	20.2		
No	115	29	25.2		
Milking hygiene				1.434	0.231
Washed	5	0	00.0		
Unwashed	313	70	22.4		
Insects				3.010	0.083
Yes	300	69	23.0		
No	18	1	5.56		
Dogs				1.602	0.206
Yes	14	5	35.7		
No	304	65	21.4		