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Seroprevalence of Brucellosis in Saanen Goats in Khartoum North, Sudan

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Abstract

A cross-sectional study was carried out to determine the prevalence of brucellosis in Pure Saanen goats in Khartoum North. Three areas were selected in the state: Shambat, Bahri University (Veterinary Clinic) and Alkadaro. A total of 130 Saanen goats were randomly selected. Blood samples were taken and examined with Rose Bengal Plate Test (RBPT) and competitive Enzyme Linked Immune-sorbent Assay (ELISA).

The results of examination of the serum samples showed that 5 of the samples were positive (prevalence) with Rose Bengal Plate tests (3.8%), which were confirmed negative with the competitive ELISA. All the positive cases were males, of good body condition and good hygienic conditions. The statistical association between risk factors and brucellosis was done by the univariate analysis (chi square test) followed by multivariate analysis (logistic regression). With the exception of age (x2=3.731, df=1, p=0.053) all studied risk factors (educational level of the owner, pregnancy, sex, present of dogs, insemination, body condition, hygiene conditions and history of abortion) revealed no significant statistical association with brucellosis even with RBPT.

The study concluded that the prevalence of brucellosis in Saanen goats was relatively low in Khartoum North. It was recommended that more studies on potential risk factors and isolation of *Brucella* spp from goats in general and Saanen goats in particular in the Khartoum North are important for designing control policies.

Keywords: Saanen, Brucellosis, Goats, Elisa, Prevalence, Risk factors.

1. Introduction

Brucellosis, a zoonosis, is a multiorgan infectious disease. The sources of infection are sheep, cattle, goats, dogs, swine, reindeer and camels. Transmission to humans occurs from direct contact via skin abrasions and mucous membranes or from the inhalation and ingestion of contaminated animal products, such as milk products, meat and body fluids. The genitourinary system is be affected in 2–20% of the cases with brucellosis and the most common form is seen as orchitis.1–5 Up to 10% of patients systemic brucellosis relapse after antimicrobial therapy.1,2 Relapse may occur due to intracellular localization of the organisms, which protects the bacteria from certain antibiotics and host defense mechanisms (KOÇAK1,2014).

Brucella spp. is gram-negative, facultative intracellular pathogens responsible for zoonotic infections. The natural hosts of *Brucella spp*. include a wide range of mammals, including domestic farm animals and wildlife species (Boschiroli *et al.,* 2001). A distinctive feature of both zoonotic disease in humans and infections in the natural reservoirs for *Brucella spp*. is the ability of bacteria to persist in host tissues over a period of years (Ariza *et al*., 1995; Margolis and Collins, 1997; Tittarelli *et al.,* 2005). In infected humans and animals, *Brucella spp.* have been identified in tissues of the reticulo-endothelial system (RES), including bone marrow, lymph nodes and spleen, suggesting that these are important sites of bacterial persistence during chronic infection (Roux1*et al,* 2007).

The species of *Brucella* and their major hosts are *B. abortus* (cattle), *B. Melitensis* (goats), *B. suis* (swine) and *B. ovis* (sheep). *B. abortus* also causes infection in horses and is commonly found in chronic bursal enlargements as a secondary invader rather than a primary pathogen (Radostits *et al*., 2000).

Brucellosis is a severe zoonosis in North African countries and the Near East causing economic and livestock losses and affecting industrial production. In these regions *Brucella melitensis,* the predominant species in goats and sheep has spread to other animals such as cattle and camels. *B. melitensis* is also the dominant type in humans. Consumption of unpasteurized milk and milk products from cows, small ruminants or camels is considered to be the main route of infection as well as an occupational hazard . In the North African region, as in sub-Saharan countries, social and economic factors play a major role in the spread of brucellosis (Ahmed *et al.,* 2010).

Goats are the primary reservoir of *Brucella melitensis*, which is the most pathogenic species for humans and animals, rather than other *Brucellae*. Furthermore, the goat production is an important source of food for self-consumption and economic resources for smallholders in rural areas of the world (Acosta-Conzalez *et al.*,2009).

Sudan is characterized by a wide range of climatic zones, and accordingly cattle, goats and sheep are distributed. a survey in 2010 done by the Ministry of Animal Resources and Fisheries estimated a total population of 76 million cattle 52 million sheep and 43 million goats in the country (Behnke and Osman 2012).

The goat is the most widely distributed animal in the Sudan. This mainly because it is easy to handle and can look after itself very well indeed. Women and children are the major handlers of goats in the Sudan. Mason and Maule (1960) have indicated that indigenous goats can be classified into 4 breeds groups: Nubian, Desert, Nilotic, Mountain breeds. However, there were a few introduced foreign breeds, which have adapted very well under Sudan conditions when crossed with local breeds. These are Toggenberg, Swiss breed, Anglo- Nubian and

Saanen, which is the most recent introduction from Syria and is spreading very fast (FAO, 2010).

Transmission and spread of brucellosis is affected by variety of factors and knowledge of these is essential to successful control policy (Reviriego *et al*., 2000; Bikas *et al*., 2003; Minas *et al*., 2004). In addition to its effects on human and animals, brucellosis causes economic losses through abortions, stillbirths, subsequent delayed or permanent infertility, prolonged calving interval and loss of milk and meat production. The disease poses a barrier to trade of animals and animal products. It has a wide socioeconomic impact especially in countries where rural income relies to a large extent on livestock breeding and dairy production (Zinsstag *et al*., 2005). The disease can also have an impact on exports and constrains efforts to improve breeding. From 1999 to 2005, a total of ships carrying 40,428 sheep, 5,334 goats and 2,298 camels were rejected by Saudi authorities because of detection of some cases positive for brucellosis and that had resulted in heavy financial losses and bankruptcy of some animal traders (Musa *et al*., 2008).

In the Sudan the disease was first reported from a dairy farm in Khartoum (Bennett, 1943). Thereafter, many investigators isolated the organism from different sources in different localities in the country (Daffalla, 1962, Shigidi and Razig 1971 – 1973, Musa and Mitchell; 1985; Musa; 1995, Raga, 2000, Rias, 2004 and Gasim, 2009). 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 *et al;* 2001).

The objectives of this study were: to determine the prevalence of Brucellosis in Saanen goat by serological tests and to investigate the risk factors associated with the Brucellosis in Saanen goat.

2. Materials and methods

2.1 Study area

Study area chosen is Khartoum state, located in North Eastern part of the center of Sudan. The state is between latitudes 15.8º and 16.45º north, longitudes 31.5º and 34.45º east, it is covers 20’736 km. The climate is semi-desert, dry and hot in summer (maximum temperature is 47.1º C and minimum temperature is 22.7º C) the average rainfall is 150 mm^2 per year.

2.2 Study design

A cross-sectional study was carried out from June to November 2018, to determine the prevalence of Brucellosis in Pure Saanen goats in Khartoum North. Three areas were selected in the state: Shambat, Bahri University (Veterinary Clinic) and Alkadaro. A total of 130 Saanen goats were randomly selected. Blood samples were taken and examined with Rose Bengal Plate Test (RBPT) and competitive Enzyme Linked Immune-sorbent Assay (ELISA).

2.3 Collection of samples

2.3.1 Serum samples

One hundred and thirty blood samples were collected from Saanen goats from Alkadru, Alhalfia, Bahare university, and Shambat by venipuncture. The skin at the site of venipuncture was shaved and swabbed with 70% alcohol and allowed to dry, 5 ml of blood was been taken by needle and plain Vacutainer tube (OIE, 2008). The blood tubes were placed in racks and left to stand at ambient temperature for 1 to 2 h in slanting position until the clot began to contract. The racks bottles were placed in a refrigerator at 4°C. After overnight, sera decanted or removed with a pipette in

Eppendorf tubes, labeled and preserved in an ice box which is transported to laboratory.

2.4 Serological methods

2.4.1 Rose Bengal plate test (RBPT)

2.4.1.1 Material and reagent

Rose Bengal antigen
Micropipette test
Plate shaker
Mixing rods

Brucella colored antigen used in this test was obtained from Division of *Brucella* research in Veterinary Research Institute (VRI) Soba, the antigen and the method were done as described by Alton (1985). The antigen and the serum samples were removed from the refrigerator to room temperature and shaken properly before use. Equal quantity of serum sample and (RBPT) antigen (25 µl) were taken on an enamel plate, mixed thoroughly with metal stick and rotated clockwise and anti-clockwise. The result was read immediately after 4 min. definite agglutination was considered as positive reaction.

2.4.2 Enzyme linked immune-sorbent assay (cELISA):

2.4.2.1 Competitive ELISA:

2.4.2.1.1. Kit contents and instructions

Only sera which were positive by RBPT were selected by cELISA and all the following were included in the kit before proceeding. Refrigerate kit was immediately on arrival and store conjugate at -20°C. Plates: Plates pre-coated with *B. melitensis*, LPS antigen and Lid. Diluting buffer: Tablets of phosphate buffered saline (PBS), phenol red Indicator and Tween 20. Wash solution: Na2HPO4 and Tween20.Conjugate: As supplied (store at -20°C). Chromogen: OPD tablets (Toxic).Substrate: Urea hydrogen peroxide tablets (irritant).Stopping solution: Citric acid (irritant).Control: Positive serum and negative serum.

2.4.2.1.2. Equipment required:

Microtitre plate reader with 450 nm filter, Single and multichannel variable volume pipettes, disposable tips for the foregoing, Reagent troughs for multichannel pipetting. 10 L container for wash fluid, 4 ± 3°C refrigerator, rotary shaker, capable 160 Rvs/Min (or a 37 ± 3°C incubator), microtitre plate shaker, sterile distilled or de ionized water, bottles tubes and beakers for storage of sera and reagents, absorbent paper towels, and freezer for storage of conjugate. The microtitre plate reader is not essential as an assessment of the results can be performed visually. The use of an incubator and or shaker is preferable, but by adapting the method their use is not essential.

2.4.2.1.3. Reagent preparation

1. Reagents provided were sensitive to changes in temperature and light. They must be prepared and stored as per instructions if they are effective in the test.
2. Very clean glassware and pure distilled water were vital for the preparation and storage of reagents.

2.4.2.1.4. Diluting buffer

Prepare diluting buffer by adding 5 tablets of PBS, 0.5 ml phenol red indicator and 250 µl of Tween 20 to 500 ml-distilled water. The pH must be between 7.2 and 7.6 - phenol red will turn yellow below pH 7.2 and violet above pH 7.6. The buffer should be discarded if this happens and store at 4 ± 3°C. Do not keep for more than 1 month.

2.4.2.1.5. Wash solution

Prepare the wash solution by adding the contents of the ampoule of Na2HPO4 (0.14 g) and 1 ml of Tween 20 to 10 L of distilled water. This can be stored at room temperature (21 ± 6°C). Do not keep for more than 1 month.

2.4.2.1.6. Conjugate

Prepare the conjugate by adding 1 ml of the content of the conjugate ampoule to 11 ml of diluting buffer to give 12 ml of the conjugate. Once the conjugate has been prepared according to instructions on the ampoule, it must not be stored.

2.4.2.1.7. Stopping solution

Prepare the stopping solution by diluting the contents of the ampoule of citric acid (2 ml) with 38 ml of distilled water and store at 4 ± 3°C. Do not keep for more than 1 month.

2.4.2.1.8. Controls

Reconstitute each of the positive and negative control samples included in the kit with 1 ml sterile distilled water. Allow to stand until an even suspension is obtained. Ensure the entire contents are completely resuspended before use. Store at 4 ± 3°C. If the control samples are to be kept more than 1 week, store at -20 ± 5°C in aliquots.

2.4.2.1.9. Procedure

1. We were prepared the conjugate solution. Diluted to working strength with diluting buffer according to instructions on the ampoule label.
2. We were Added 20 µl of each test serum per well. Leave columns 11 and 12 for controls.
3. We were Added 20µ1 of the negative control to wells All, A12, Bl1, B12, C11 and C12.
4. We were Added 20 µl of the positive control to wells F11, F12, G1l, G12, H11 and H12.
5. The remaining wells had no serum added and act as the conjugate controls.
6. Immediately, we were dispensed into all wells 100 µl of the prepared conjugate solution. This gives a final serum dilution of 1/6.
7. The plate was then vigorously shake (on the microtitre plate shaker) for 2 min in order to mix the serum and conjugate solution. the plate was Covered with the lid and incubated at room temperature (21 ± 6°C) for 30 min on a rotary shaker, at 160 revs/min.
8. We were shaking out of the contents of the plate and rinse 5 times with washing solution and then thoroughly dry by tapped on absorbent paper towel.

9. Then we were Switched on microplate reader and allowed the unit to stabilize for 10 min.
10. Immediately before use we were prepared the substrate and chromogen solution by dissolved one tablet of urea H2O2 in 12 m1 of distilled water. When dissolved we were added the OPD tablet and mix thoroughly. That was took a few minutes, the use of a magnetic stirrer greatly increased the speed with which it dissolves. We were Added 100 µl to all wells. This solution cannot be stored**.**
11. We were Leave the plate at room temperature for a minimum of 10 min and a maximum of 15 minutes.
12. We were Slowed the reaction by added 100 µl of stopping solution to all wells.
13. We were Remove condensation from the bottom of the plate with absorbent paper towel and we were Read plate at 450 nm.

2.4.2.1.10. Analysis of results

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 the optical density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to or below this value should be regarded as being positive.

2.4.2.1.11. Plate rejection

The results considered invalid if any of the following apply;
1. The mean OD of the 6 negative control wells is less than 0.70.0. (The optimal mean negative OD is 1.000).
2. The mean OD of the 6 positive control wells is greater than 0.100.
3. The mean OD of the 4 conjugate control wells is less than 0.700 (the optimal mean conjugate control OD is 1).
4. The binding ratio is less than 10.

$$\text{Binding ratio} = \frac{\text{Mean of 6 negative control wells}}{\text{Mean of 6 positive control wells}}$$

2.5. Data analysis

The data collected from the field and the laboratory result were stored in Microsoft excel spreadsheet program, and the statistical analysis was performed using SPSS version 16.0 soft program.

3. Result

3.1 serological test result:

Out of the 130 serum samples 5 (3.8%) positive for brucellosis by the Rose Bengal plate (RBPT) and there were no serum samples positive by competitive enzyme linked immunosorbent assay (c ELISA) when was used as confirmatory test.

Table 1: Result of serological testing of serum samples for brucellosis in saanen goats.

Serological test	Total No of sera screened	No. of positive (%)
RBPT	130	5(3.8%)
cELISA	5	0(0%)

Table 2: The univariate analysis for risk factors of Brucellosis in Saanen goats examined in Khartoum north using the Chi-square test.

Risk Factor	No. of tested Samples	No. of positive Samples	% Positive (Prevalence)	d.f	X^2	P.value
Education of owner						
Educated	117	5	4.3%	1	.000	1.000
Non educated	13	0	0%			
Hygiene of environment						
Clean	130	5	3.8%	1	0	0
Dirty	0	0	0%			
Insemination						
Artificial	8	1	12.5%	1	.133	.715
Natural	122	4	3.3%			
pregnancy						
Pregnant	40	0	0%	1	1.053	.305
Non pregnant	90	5	5.5%			
Age						
Young	68	0	0%	1	3.731	0.053
Old	62	5	8.1%			
Present of dog						
Present	121	5	4.2%	1	.000	1.000
Absent	9	0	0%			
Body condition						
Good	130	5	3.8%	1	0	0
Poor	0	0	0%			
Sex						
Male	111	5	4.5%	1	.089	766
Female	19	0	0%			
Abortion						
Aborted animals	5	0	0%	1	.000	1.000
No abortion	125	5	4%			

*P ≤ 0.05 was consider as significant.
*df =degree of freedom.

3.2 The univariate analysis for risk factors of Saanen brucellosis in Saanen goats examined in Khartoum north using the Chi-square test:

In the univariate analysis using chi square, age (x2=3.731, df=1, p=0.053) was only significantly associated with brucellosis infection. However, owner education, Hygiene condition, insemination, pregnancy, Present of dog, body condition, sex and abortion were not significantly associated with Saanen brucellosis in Table (2).

3.2.1 Educational level of the owner:

No significant association were found between brucellosis and educational level of the owner (*x2* = .000 , P.value = 1.000) ,the total number of Saanen goats those were examined for educated owner during this study were 117 out of them 5 (4.3%) were have Saanen brucellosis table (2).

3.2.2 Hygiene condition:

Statistical analysis was not done for hygiene of environmental because all Saanen goats in present study were having clean environment in table (2).

3.2.3 Insemination:

No significant association were found between brucellosis and insemination (x2= .133, P.value = .715), the total number of Saanen goat examined which artificially inseminated were 8 out of them 1 (12.5%) were have brucellosis infection ,among 122 naturally inseminated goats 4 (3.3%) were have Saanen brucellosis in table (2).

3.2.4 Pregnancy:

No significant association were found between brucellosis and pregnancy (x2= 1.053 , P.value = 0.305) , the number of non-pregnant Saanen goats examined were 90 out of them 5 (5.5%) were have Saanen brucellosis in Table (2).

3.2.5 Age:

significant association were found between brucellosis and age (x2= 3.731, P.value = 0.053) , the number of old Saanen goats examined were 62 out of them 5 (8.1%) were have Saanen brucellosis in table (2) .

3.2.6 Present of dog:

No significant association were found between brucellosis and present of dog (x2 =.000 , P.value1.000 =) the total number of Saanen goat examined with the present of dog were 121 out of them 5 (4.2%) were have Saanen brucellosis in table (2).

3.2.7 Body condition:

Statistical analysis was not done for body condition because all Saanen goats in present study were having good body condition table (2).

3.2.8 Sex:

No significant association were found between brucellosis and sex (x2=.089 , P.value = 766) , the total number of male Saanen goats examined were 111 out of them 5 (4.5%) were have Saanen brucellosis in table (2).

3.2.9 Abortion:

No significant association were found between brucellosis and abortion (x2= .000, P.value = 1.000) the total number of non-aborted Saanen goats examined were 125 out of them 5 (4%) were have Saanen brucellosis in table (2).

Table 3: The multivariate analysis for risk factor of Saanen brucellosis in Saanen goats examined in Khartoum north using logistic regression:

Risk Factor	No. of tested Samples	No. of positive Samples	% Positive (Prevalence)	Exp B	(95% CI - Exp B) Lower	Upper	P.value
Education level of the owner							
Educated	117	5	4.3%	.000	.000	.	.999
Non educated	13	0	0%				
Insemination							
Artificial	8	1	12.5%	.516	.046	5.840	.593
Natural	122	4	3.3%				
Pregnancy							
Pregnant	40	0	0%	.000	.000	.	.998
Non pregnant	90	5	5.5%				

Age							
Young	68	0	0%	753464.255	.000	.	.997
Old	62	5	8.1%				
Present of dog							
Present	121	5	4.2%	721481.870	.000	.	.999
Absent	9	0	0%				
Sex							
Male	111	5	4.5%	.500	.000		1.000
Female	19	0	0%			.	
Abortion							
Aborted animals	5	0	0%	.000	.000	.	.999
No abortion	125	5	4%				

*P ≤ 0.05 was considered as significant.
*C.I.= confidence interval.
*Exp (B) = exponent B, representing the odds ratio.

3.3 The multivariate analysis for risk factor of Brucellosis in Saanen goats examined in Khartoum north using logistic regression:

In multivariate analysis, none of the factors (educational level of the owner, pregnancy, sex, age, present of dogs, insemination and history of abortion) found to be significantly associated with Saanen brucellosis in table (3).

Figure 1: RBPT association with Education of owner, Hygiene of environment, Dog present, Insinuation and Body condition.

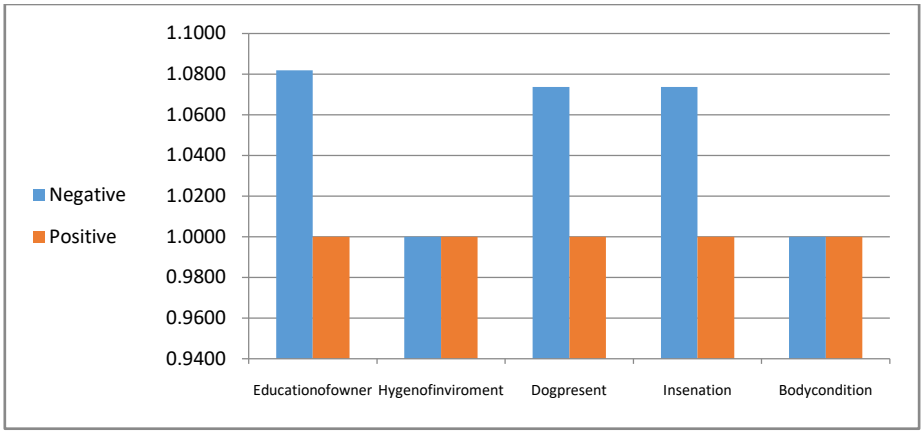


Figure 2: RBPT association with Abortion, pregnancy, sex and age.

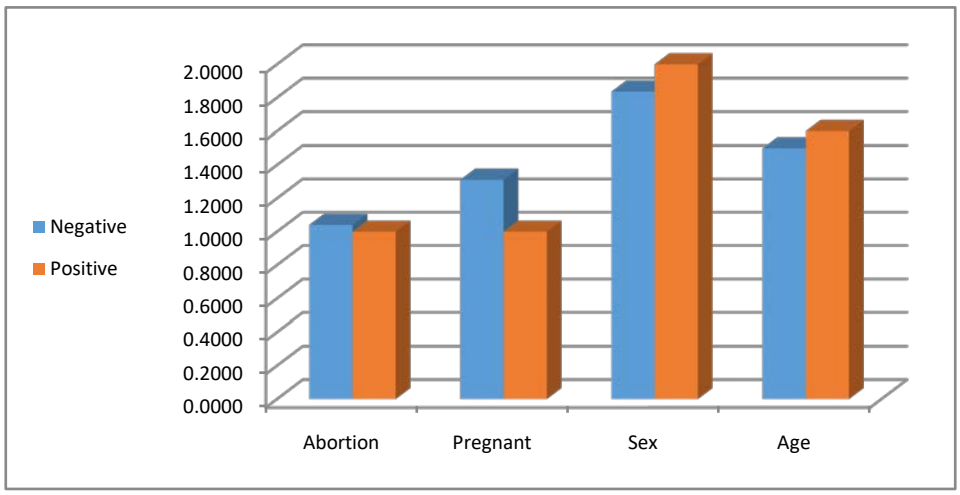
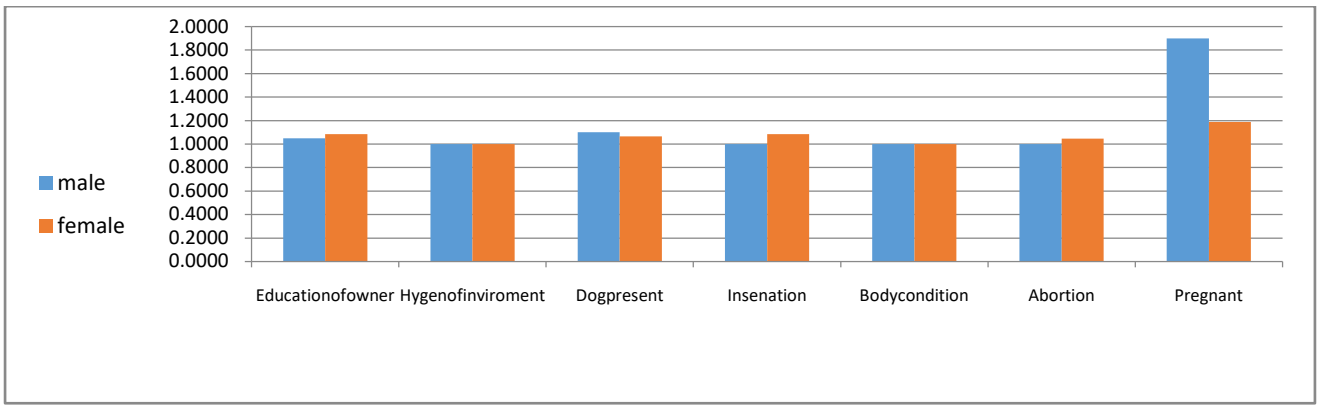


Figure 3: Association of sex with Education of owner, Hygiene of environment, Dog present, Insinuation, Body condition, Abortion and Pregnant.



Discussion

This study revealed that the overall prevalence of saanen goats Brucellosis in Khartoum north State was 3.8%by RBPT and all cELISA results were negative and all the positive cases were males of good body condition (100%), 1.4% were in the age group of 1-2 years old while 2.4% were in the age group of 3-7 years old. Which means that the prevalence of Brucellosis in saanen goats was relatively low. This may be due to the good health status of these animals and there high selectivity by owners. The negative results of saanen goats Brucellosis may be explained by the fact that the cELISA is a more specific test than RBPT, but sometimes it fails to detect infected animals which positive by RBPT. Corbel (2006).

The low prevalence rates of brucellosis among tested animals may be attributed to several factors that might reduce the spread of the disease. These factors include the climatic conditions of the Sudan which characterized by persistence of the sun light most hours of the day, dry desert weather and low humidity. El Sanousi (2012). In addition to that, the raising of Saanen goats in Khartoum north state is mostly done by educated people who are carful in biosecurety, selectivety and introduction of new purchases which prevents Saanen goats from disease. The recommended serological tests for *Brucella* detection in ruminants are

complement fixation test (CFT), Rose-Bengal test (RBT), sero-agglutination test (SAT) and competitive enzyme-linked immunosorbent assay (c-ELISA) (OIE, 2018).

The prevalence rate observed in this study using RBPT was lower than those rate reported by several authers: Osman (2013) reported 11.4%, ELAnsary (1999), 4%, Rayas (2004), 0.3%, Ahmed (2004), 0.45, Acosta-Conzalez et al (2009), 6.79%, Asharegie et al (2011), 4.2%, Ashenafi et al(2007) , 5.8%, Rahman et al (2011), 3.15% and AKbarmehr and Ghyumirad (2011),5%.It is difficult to explain these differences in the results obtained by RBPT. However, the goats tested in previous study were in different parts of the country and from different numbers and breeds, and this might have an effect on the result. The prevalence rate observed in this study was higher than the result reported by earlier workers. El Sawi et al. (1981) found that 0.65% of goats tested were positive, fayza et al. (1990) examined 2233 sera from goats destined for export and found that only 0.1% were positive, Ginawi (1997) screened 190 goats sera and found them all negative (0%), and Hayfa et al. (2001) examined 1000 sera from goats and found that only 1.5% were positive. Generally it can be concluded that the prevalence of brucellosis in saanen goats in Khartoum north state was very low as only 5 sample were found positive by the RBPT and these sample were not confirmed by cELISA. In other ruminant brucellosis (sheep) Brucellosis was found by Ginawi (1997) who examined 500 heads using RBPT, SAT and CFT. But he did not recorded any positive result .its recommended that more sample be examined from Saanen goats using more test to reach the definitive conclusion about the prevalence of brucellosis in Saanen goats in Khartoum north.

According to this study it could be concluded that, the prevalence of brucellosis in Saanen goats was very low in Khartoum north. It is recommended that, sample size should be representatives in further researches and more surveys are required to investigate the Saanen brucellosis in the states.

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