Sero-Prevalence of Brucellosis in Sheep in El-Gadarif State

Mohammed Abdalkarim Abdalla[1], Enaam Mohamed El-Sanousi[2], Yassir Adam Shuaib[3], Hatim Hamad Ibrahaem[2], Khalid Musa Fadle-Al-Mola[2], Saad El-Tiab Mohamed-Noor[3], Siham Elias Suliman[3], Salah Hassan Idris[2] and Mohamed Abdelsalam Abdalla[3]*

[1]*Kassala Center for Quarantine, Inspection and Vaccination, Federal Ministry of Animal Resources and Fisheries, Kassala, Sudan*
[2]*Veterinary Research Institute, Soba, Khartoum, Sudan*
[3]*College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum North, Sudan*

*Corresponding Author: Mohamed A Abdalla, Professor, College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum North, Sudan.

Abstract

This survey was conducted in El-Gadarif state in the period from March 2016 to March 2017 to investigate the sero-prevalence of brucellosis in sheep. A total number of 558 serum samples were collected from sheep form five localities. The collected samples were first screened for presence of anti-*brucella* antibodies by Rose Bengal Plate test (RBPT). In a second step, samples that were RBPT positive besides to some randomly selected RBPT negative (n = 144) were subjected to indirect enzyme linked Immunosorbent assay (i-ELISA). Overall, a percentage of 7.4% (n = 41) of the serum samples showed RBPT positive results, of which the i-ELISA confirmed the positivity of 25 (4.5%) samples. The other 10 i-ELISA positive samples were RBPT negative. It can be concluded that brucellosis is prevalent in sheep in El-Gadarif. Adopting of an all-year-round surveillance system to enable early detection of *Brucella* infections is highly recommended as it will assist in controlling and managing the disease.

Keywords: Sero-prevalence; Brucellosis; Sheep; El-Gadarif

Introduction

Brucellosis is an infectious disease of livestock as well as humans [1]. It is a result of infection with bacteria from the genus *Brucella* [1,2]. This genus contains more than ten different species that are gram-negative, non-spore forming, non-motile, non-capsulated, aerobic and facultative intracellular coccobacilli or short rods [1-4]. In Sheep, *Brucella melitensis* and *B. ovis* are the main causative agents of brucellosis and their infection often leads to abortions in late pregnancy, stillbirths, and birth of weak offspring besides to reductions in milk yield [1].

In addition to its public health significance, brucellosis results in huge economic losses to sheep production and industry [1,5-7]. These losses are basically due to abortions and fertility problems. Furthermore, it poses a barrier to national and international trade of sheep [6,7]. Hence, control of brucellosis is a priority in many countries and depends on application of strict hygiene measures and vaccination programs. The main objective of vaccination is to reduce the prevalence of the disease to a level where eradication by test-and-slaughter is achievable [6,7]. Many developed countries have successfully controlled and eradicated brucellosis and are officially brucellosis-fee now [6-9]. However, it is still a great health problem and prevalent in some developing countries in Sub-Saharan Africa e.g. Sudan [10-14].

The disease was detected during the 1940s for the first time in the Sudan. Since then, it has frequently been reported in different parts of the country in domestic and wild animals and in humans too [10-12]. Very few studies did investigate brucellosis in goats, cattle, and camels in El-Gadarif [10,13,14]. However, epidemiology and prevalence of brucellosis in sheep in El-Gadarif are neither very well investigated nor understood. Therefore, this survey is conducted to determine the sero-prevalence of anti-*brucella* antibodies in sheep in El-Gadarif state in Eastern Sudan.

Materials and Methods

Study area

El-Gedarif state is one of the states of Eastern Sudan. It is located between longitudes 33°30' and 36°30' East and latitudes 12°40' and 15°46' North and covers a total area of 75,263 km^2. It falls within the Sudano-Sahelian climate zone of Africa and shares international border with Ethiopia from the East and national borders with Kassala and Khartoum states from the North, with El-Gezira state from the West and with Sinnar state from the South. Pastoral nomadism is the main animal husbandry system in El-Gedarif. The soil of the state is very fertile enabling massive agricultural activities in rainfall season. Therefore, El-Gedarif is very rich in livestock resources that are integrated in the agricultural system. The animal population in El-Gadarif is comprised of about 1,454,222 head of cattle, 3,688,218 head of sheep, 884,087 head of goats, and 880,162 head of camels [15].

Sampling strategy and study design

As described by Thrusfield [16], this cross-sectional survey was conducted in the period from March 2016 to March 2017. Five out of the ten localities in El-Gadarif state were conveniently selected, namely: Basunda, Gallabat Sharqia, Gallabat Gharbia, Baladiat El-Gadarif and Al-Fashaqa. Within the selected localities, smaller administrative units and/or villages as well as sheep flocks and individual animals were randomly and/or conventionally selected and sampled [16].

Sample size and collection

The sample size (n) to determine the prevalence of anti-*brucella* antibodies in sheep was calculated using the standard formula of Thrusfield [16]. The required sample size was determined to be 558 sheep. Samples distribution between localities was as follows: 36.2% (n = 202) samples were from Basunda, 17.9% (n = 100) from Gallabat Sharqia, 8.3% (n = 46) from Gallabat Gharbia, 17.9% (n = 100) from Baladiat El-Gadarif, and 19.7% (n = 110) from Al-Fashaqa.

Whole blood samples were taken using needles and plain vacutainer tubes from the jugular veins of the selected sheep. Serum samples were separated and kept at -20°C until used as recommended by OIE [17].

Laboratory tests

Rose Bengal plate test

The Rose Bengal Plate test (RBPT) was performed and interpreted according to the guidelines of OIE [17]. Any degree of agglutination was considered as positive reaction, whereas no reaction, i.e. failure to develop agglutination, was considered as negative and was an indication of the absence of anti-*brucella* antibodies in the sample.

Indirect enzyme linked Immunosorbent assay

An indirect enzyme linked Immunosorbent assay (i-ELISA) was performed according to the manufacturer's instructions as well as the calculation of the percentage positivity (PP) values (SVANOVIR® *Brucella*-Ab i-ELISA, Uppsala, Sweden).

Statistical analysis

The Statistical Package for Social Sciences (SPSS) for Windows® version 20.0 (SPSS Inc., Chicago, Illinois) was used for all appropriate statistical analyses. Descriptive statistics of the variables were obtained including frequencies and prevalence.

Results

Overall, 7.4% (n = 41, 95% CI between 5.5 and 9.8) of the serum samples had anti-*brucella* antibodies using RBPT. These RBPT-positive samples plus a number of randomly selected negative reactors (n = 144) were tested by i-ELISA. Out of the 41 RBPT-positive reactors, the i-ELISA confirmed the positivity of 25 (4.5%, 95% CI between 3.1 and 6.5) samples. The remaining 10 i-ELISA positive samples were RBPT negative.

Discussion

Brucellosis is consistently ranked among the most economically important zoonoses with huge production losses that may reach up to US $ 2 million per year in some parts of the world [5,18]. In sheep, brucellosis has been controlled and eradicated in many developed countries, nevertheless, it remains a great health problem and endemic in many poor countries [9]. *B. melitensis*, which is the main causative agent of brucellosis in sheep, has been reported in the Sudan [4]. However, its epidemiology is not very well understood in El-Gadarif state [10,13,14].

The present study was conducted to determine the seroprevalence of brucellosis in sheep in El-Gadarif state. A number of 41 (7.4%) of the investigated serum samples had anti-*brucella* antibodies using RBPT. This finding is lower than the findings of Khuzaima., *et al.* [14] and Shuaib., *et al.* [19] who reported that around 10% of sheep and goats samples investigated for brucellosis in Eastern Sudan as positive reactors. However, it is higher than the sero-prevalences reported in different parts of the Sudan previously (0.7% to 3.4%) [20-24]. In African and Asian countries the average prevalence of brucellosis in small ruminants was between 0.0% and 88.8% [25]. There are many reasons that can lead to variations in the sero-prevalence of brucellosis among sheep such as, for example, i) quality of veterinary services, ii) animal husbandry, iii) geographical locations, and iv) breed of animal [1]. Abdelnassir [10] indicated that the prevalence of brucellosis in El-Gadarif state is primarily due to the lack of proper control and eradication programmes as well as because of mixing of different animal species (sheep, goats, and cattle) in one herd/flock. The little knowledge of animal owners/herders on brucellosis could probably be another important factor for spreading of brucellosis. When animal owners/herders screen their animals for brucellosis, positive reactors are not usually culled out but are rather kept within the herd. This increases the chances of transmission among animals. Out of the 41 RBPT-positive reactors, the i-ELISA confirmed the positivity of 25 samples. Therefore, the corrected prevalence of anti-*brucella* antibodies in sheep in El-Gadarif state was 4.5%. The other 10 i-ELISA positive samples were RBPT negative. These RBPT false negative results can be due to the prozoning or the hook effect i.e. lack of agglutination at high concentrations of antigen or antibodies. Alternatively, the false negative reactions can be due to a small clump size in the serum samples and low anti-*brucella* antibody titers [26].

The finding of this survey implies that brucellosis in sheep may represent a potential hazard to other animal species in El-Gadarif. This is because some animal owners/herders do keep different animal species in one herd/flock. This increases the likelihood of between-species transmission. Moreover, there is also a risk of brucellosis transmission from sheep to humans including veterinarians, butchers, and herders.

The use of RBPT and i-ELISA in a serial manner for screening of the collected samples and for confirming the positive results of the screening test reduced probability of false positive results. This is the main strength of this survey. However, using neither culture nor molecular tests to detect *Brucella* pathogens besides to failure to investigate management risk factors are the limitations of the study.

Conclusion

In conclusion, brucellosis is prevalent in sheep in El-Gadarif. Studies investigating potential risk factors that enhance spreading and transmission of brucellosis in sheep and other animal species are warranted. Adopting of an all-year-round surveillance system to enable early detection of *Brucella* infections is highly recommended. It will assist in the control and management of the disease.

Conflict of Interest

No conflict of interests.

Bibliography

1. Radostits OM., *et al*. "Disease caused by Brucella spp. A Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses". 10th Edition, ELBS Bailliere Tindall, London, UK (2007): 870-871.

2. Godfroid J. "Brucellosis in livestock and wildlife: zoonotic diseases without pandemic potential in need of innovative one health approaches". *Archives of Public Health* 75 (2017): 34.

3. Watmore AM. "Current understanding of the genetic diversity of brucella, an expanding genus of zoonotic pathogens". *Infection Genetics and Evolution* 9.6 (2009): 1168-1184.

4. Eman MA., *et al*. "Seroprevalence and risk factors of caprine brucellosis in Khartoum state, the Sudan". *Veterinary World* 11.4 (2018): 511-518.

5. McDermott J., *et al*. "Economics of brucellosis impact and control in low-income countries". *Revue Scientifique et Technique* 32.1 (2013): 249-261.

6. Samadi A., *et al*. "Ovine and Caprine Brucellosis (Brucella melitensis) in Aborted Animals in Jordanian Sheep and Goat Flocks". *Veterinary Medicine International* (2010): 458695.

7. Rubach P., *et al*. "Brucellosis in low-income and middle-income countries". *Current Opinion in Infectious Diseases* 26.5 (2013): 404-412.

8. Refai M. "Incidence and control of brucellosis in the Near East region". *Veterinary Microbiology* 90.1-4 (2002): 81-110.

9. Muma J., *et al*. "Prevalence of antibodies to Brucella spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in livestock-wildlife interface areas of Zambia". *Tropical Animal Health and Production* 38.3 (2006): 195-206.

10. Abdelnassir MT. "Detection of Antibodies against Bovine Brucellosis in ElHawata area, ElGadarif State". M.V.Sc. Thesis, University of Khartoum, Sudan (2007).

11. Musa MT El Sanousi., *et al*. "Brucellosis, a challenge to veterinarians in Africa: The situation of the disease in the Sudan". The proceeding of the first scientific conference. Khartoum, Sudan (2008): 45-56.

12. Badri AM., *et al*. "Sero-Prevalence and Molecular Detection of Brucellosis among Febrile Patients in West Darfur State, Sudan". *Molecular Biology* 7.1 (2018): 204.

13. Mahasin MA., *et al*. "Sero-Prevalence of Anti-Brucella Antibodies in Camels in El-Gedarif State: a short communication". *Scholars Journal of Agriculture and Veterinary Sciences* 4.11 (2017): 461-463.

14. Khuzaima BA., *et al*. "Sero-prevalence of anti-brucella antibodies in goats in El-Gedarif state, Eastern Sudan". *ARC Journal of Animal and Veterinary Sciences* 4.1 (2018): 1-8.

15. Anon. Department of Statistic and Information. Annual Report. Ministry of Animal Resources, El-Gadarif, El-Gadarif state, the Sudan (2012).

16. Thrusfield M. "Veterinary Epidemiology". United Kingdom, Black Well Science ltd, Ed. (3), Chapter 15 (2007): 220-221.

17. OIE, Terrestrial Manual Chapter 2.4.3. Bovine brucellosis. World Organization for Animal Health (OIE), Paris, France (2012).

18. Godfroid J., *et al*. "From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis". *Veterinary Research* 36.3 (2005): 313-326.

19. Shuaib YA., *et al*. "Sero-prevalence of sheep brucellosis in three different locations in Kassala state: a short communication". *Journal of Dairy, Veterinary and Animal Research* 7.2 (2018): 53-57.

20. Omer MM., *et al*. "Survey of brucellosis among sheep, goat, camels, and cattle in Kassala Eastern Sudan". *Journal of Animal and Veterinary Advances* 6.5 (2007): 635-637.

21. Abdallah AA., *et al*. "Seroprevalence and Risk Factors of Brucellosis in Sheep in North Kordofan State, Sudan". *IOSR Journal of Agriculture and Veterinary Science* 8.1 (2015): 31-39.

22. Osman MA., *et al*. "Detection of Brucellosis in sheep intended for export and local slaughter in Khartoum State, Sudan". *African Journal of Microbiology Research* 6.39 (2012): 6805-6810.

23. Gumaa MM., *et al*. "Seroprevalence of brucellosis in sheep and isolation of Brucella abortus biovar 6 in Kassala state, Eastern Sudan". *Revue Scientifique et Technique* 33.3 (2014): 957-965.

24. Solafa ZE., *et al*. "Prevalence and risk factors of ruminants brucellosis in Jabel Aolia locality, Sudan". *Sudan Journal of Science and Technology* 15.2 (2014): 60-72.

25. Franc K., *et al*. "Brucellosis remains a neglected disease in the developing world: a call for interdisciplinary action". *BMC Public Health* 18.1 (2018): 125.

26. Saxena H., *et al*. "Simple solutions to false results with plate/slide agglutination tests in diagnosis of infectious diseases of man and animals". *MethodsX* 2 (2015): 345-352.