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The seroprevalence and Geographic Distribution of Camel Brucellosis in Kordofan States, Western Sudan

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Abstract

Brucellosis is a highly contagious zoonotic disease that affects both humans and wildlife. It is one of the most commonly neglected diseases worldwide and can infect a wide range of domestic animals, including ruminants such as camels, as well as various wild species. This disease poses significant socioeconomic concerns. This study investigates the distribution of brucellosis in camels in Sudan's Kordofan States and compares several diagnostic tests, including a modified Rose Bengal Plate Test (mRBPT), Buffered Plate Agglutination Test (BPAT), Serum Agglutination Test (SAT), and a competitive Enzyme- Linkimmunosorbent Assay. A total of 388 apparently healthy camels were sampled to determine the seroprevalence of brucellosis. Forty-three (11.08%), 41 (10.56%) and 30 (7.73%) were found positive by (mRBPT), (BPAT) and (SAT) respectively. All serum samples were subsequently retested using (cELISA) as confirmatory test, which confirmed that 32 samples (8.24%) were positive. When comparing the screening tests to the confirmatory test, Cohen's kappa coefficients indicated poor agreement with cELISA: 16% for mRBPT, 32% for BPAT, and 31% for SAT. A kappa value of 40 or less is considered poor. This study highlights brucellosis prevalence in camels in Sudan's Western States and suggests targeted control measures. The data indicate that the mRBPT test is a more sensitive, cost-effective, and practical screening method compared to other agglutination tests.

Keywords

Brucellosis, Camel, Geographic Distribution

Introduction

The one-humped camel (*Camelus dromedarius*) plays a significant socio-economic role within the pastoral and agricultural systems of dry and semi-dry regions in Asia and Africa (Agab *et al*., 2002; Gwida *et al*., 2011). Although the total number of dromedary camels worldwide is underreported, it is estimated to be about 24 million, with 20% distributed in Asia and 80% in Africa. Somalia and Sudan host the largest populations, constituting 70% of the African camel herd (Al-Juboori and Baker, 2012). According to the Food and Agriculture Organization (FAOSTAT 2018), Sudan has the second-largest camel population in the world, following Somalia, and these animals play an essential role in the national economy.

Brucellosis, a disease caused by various species of the genus *Brucella*, is the most widely spread zoonosis globally and adversely affects animal trade (Dawood, 2008; A. Elrayah *et al*., 2015). The infection of camel herds is influenced by the *Brucella* species present in other animals sharing the same environment, as well as husbandry practices (Musa *et al*., 2008). Serological and bacteriological investigations have shown that brucellosis is endemic in Sudan, affecting cattle, sheep, goats, camels, and humans (Musa *et al*., 2008; Omer *et al*., 2010; El Sanousi, 2006).

The epidemiology of brucellosis is typically studied through the host-parasite relationship in specific populations. Factors contributing to the risk of exposure largely depend on husbandry practices, such as inter-herd transmission, animal movement, proximity to infected herds, vaccination levels, herd size, population density, and housing methods; contamination from wildlife sources should also be considered (FAO, 2010). The survival of *Brucella* in the

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environment can play a role in the disease's epidemiology. Numerous studies have investigated the ability of *Brucella* organisms to survive under various experimental and environmental conditions. Factors like temperature, humidity, and pH significantly influence the organism's ability to survive (Calfee and Wendling, 2012; Saegerman*et al*., 2010). *Brucella* are sensitive to direct sunlight, disinfectants, and pasteurization. In dry conditions, they can only survive if embedded in protein (Davies and Casey, 1973).

Diagnosis of brucellosis is based primarily on serological tests and identification (Jacques *et al*., 1998; WOAH, 2022). Diagnosing camel brucellosis can be challenging, as the disease often presents with few clinical signs, unlike its clinical course in cattle. No commonly used serological test can be considered perfect for diagnosing *Brucella* in camels, and most tests have been adapted from cattle without adequate validation (Gwida *et al*., 2012).

Due to the limited information available about brucellosis in camels and the lack of epidemiological data, this study aims to determine the occurrence, best diagnostic serological tests, and spread of brucellosis in camels in the Kordofan States of Western Sudan. To achieve these objectives, various serological tests used for brucellosis diagnosis in camels namely the Buffered Plate Agglutination Test (BPAT), modified Rose Bengal Plate Test (mRBPT), Serum Agglutination Test (SAT), and competitive Enzyme linkimmunosorbent Assay (cELISA) have been mapped and evaluated.

Materials and Methods

Study area and population

This study was conducted in the Kordofan States of North, West, and South Kordofan (Figure 1), areas known for their high density of camels and incidence of brucellosis.The total camel population in the study area was 1,766,240, which constitutes 25.4% of the national herd, with a total camel population of 4,872,000 (FAO STAT 2018).

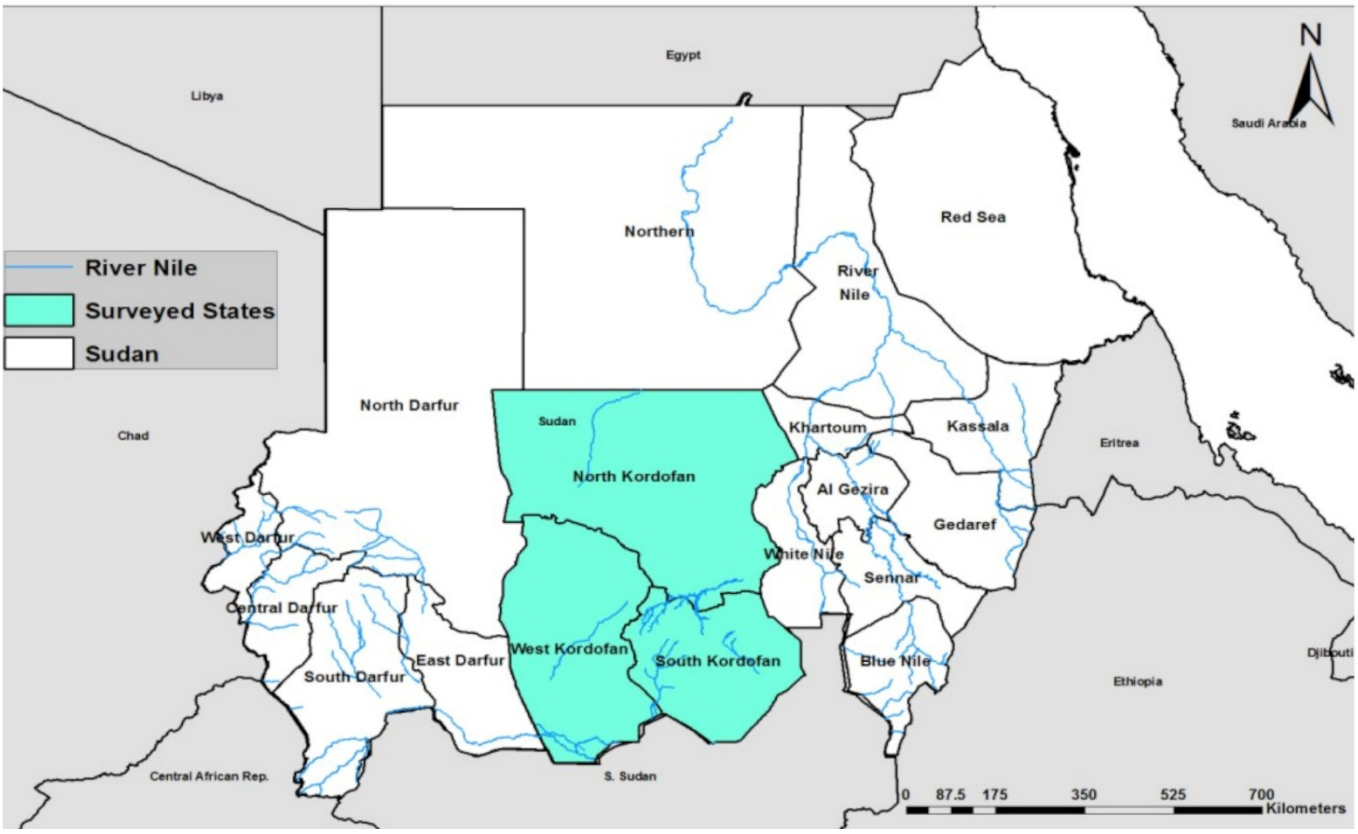

Figure 1. Kordofan States (North, West and South).

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Study Design and sample Size

A cross-sectional study was undertaken from May to December 2018 to determine the seroprevalence of camel brucellosis across the three Kordofan States. Data was collected as part of a study on the seroepidemiology of brucellosis in camel herds in five localities within these States. A multistage random sampling method was employed, based on the States, localities, and animals, as shown in Table I.

The sample size was determined using the formula for simple random sampling as outlined by Thursfield (2005). The appropriate formula for a 95% confidence level and 5% precision is:

$n = (1.96)^2 Pexp (1- Pexp)/d^2$

Where:

n = required sample size

Pexp = expected prevalence

d = desired absolute precision

The expected prevalence for this study was based on previous findings, which reported prevalence rates ranging from 1.4% to 89.5% (Musa and Shigidi, 2001). The average prevalence was estimated to be 45% and was inflated to 50% to increase the likelihood of observing and estimating the distribution of brucellosis in the States. The total calculated sample size was 388 from these States.

State	Locality	Total sample tested	RBPT %	BPAT %	SAT	cELISA %
North Kordofan	Bara	101	8 (7.92)	7 (6.93)	7 (6.93)	7 (6.93)
	Shiekan	186	30 (16.13)	29 (15.6)	20 (10.75)	22(11.82)
	Jebrt El Sheekh	20	0	0	0	0
West Kordofan	El Nehoud	51	4 (7.84)	4 (7.84)	2 (3.92)	2 (3.92)
South Kordofan	Abu Jibaiha	30	1 (3.33)	1 (3.33)	1 (3.33)	1 (3.33)
TOTAL		**388**	**43 (11.08)**	**41 10.56)**	**30 (7.73%)**	**32 (8.24%)**

Table I. Prevalence of Brucella infection as determined by mRBPT, BPAT, SAT and cELISA.

Study Animals and Collection of Serum Samples

One-humped camels (*Camelus dromedarius*) were included in this study. Blood samples were collected for serological examination from the camels, and information regarding each sampled camel, including its location, was documented. Five ml of blood were collected from the jugular vein of each of the 388 apparently healthy camels, using plain vacutainer tubes. The collected samples were allowed to clot at room temperature. The sera were then separated from the clotted blood by decanting into plastic cryogenic vials. These specimens were transported to the Central Veterinary Research Laboratory (CVRL) in Soba, Sudan, in icebox containers and were stored at -20°C until testing.

Serological Tests

Serum samples were thoroughly screened for antibodies against *Brucella* using three key serological tests: the modified Rose Bengal Plate Test (mRBPT), the Buffered Plate Agglutination Test (BPAT), and the Serum Agglutination Test (SAT). Additionally, all samples were tested using a competitive enzyme-linked immunosorbent assay (cELISA) for confirmation. It is important to note that none of the serological tests validated for use with cattle and small ruminants have been approved for use in camels yet.

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Brucella Buffered Agglutination Tests (BBATs)

These tests which include mRBPT and BPAT were conducted using standard antigens obtained from the Central Veterinary Research Laboratory (CVRL) in Soba, following the methods recommended by WOAH (2022).

Modified Rose Bengal Plate Test (mRBPT)

To improve the agglutination capability of the test and to make the results more evident, three volumes (0.075 ml) of each test serum were mixed with 0.025 ml of Rose Bengal antigen on an enamel plate and mix with spatula. The mixture was agitated for four minutes (Blasco *et al*., 1994) and then examined for the presence or absence of agglutination.

Buffered Plate Agglutination Test (BPAT)

In this method, 0.08 ml of each test serum was mixed with 0.03 ml of BPAT antigen on glass using spatula. The mixture was agitated and incubated for eight minutes in Minnesota (Alton 1988; WOAH 2022). Positive and negative controls were set up for both tests. Any degree of agglutination was considered positive, while the absence of agglutination was regarded as negative.

Serum Agglutination Test (SAT)

This is a quantitative serial dilution test used to detect classes of immunoglobulins. The test was conducted according to the method outlined in the WOAH manual (2022).

Competitive ELISA (cELISA)

The test utilized the Svanovir® *Brucella*-Ab cELISA kit according to the manufacturer's instructions, focusing on bovine brucellosis. It started with reconstituting freeze-dried monoclonal antibodies (mAb) and preparing samples by diluting them in wells with sample dilution buffer. After adding the mAb solution, the plates were sealed and incubated at room temperature for 30 minutes, followed by rinsing with PBS.

Next, a conjugate solution was added, and the plates were incubated again for 30 minutes before rinsing and drying. Substrate chromogen solution was then added, and after a final 10-minute incubation, a stopping solution was introduced to terminate the reaction.

Results were evaluated using a microplate photometer at 450 nm. Samples with a percent inhibition (PI) of 30% or higher were considered positive, while those with a PI of 30% or lower were marked as negative.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) for Windows, version 23.0 (SPSS Inc., Chicago, Illinois). The seroprevalence was determined by calculating the percentage of positivity using Buffered Plate Agglutination Test (BPAT), modified Rose Bengal Plate Test (mRBPT), Serum Agglutination Test (SAT), and competitive Enzyme linkimmunosorbent Assay. This was done by dividing the number of *Brucella* reactors by the total number of animals tested. Sensitivity and specificity were computed using cross-tabulation. The agreement between serological tests was assessed using kappa analysis.GPS was used to determine the locations where samples were collected, and these points were entered into Quantum GIS software for mapping the spatial distribution of camel brucellosis in the study area.

Results

Overall Prevalence of brucellosis

In a study of 388 serum samples from camels, brucellosis was detected in 43 samples (11.08%) using (mRBPT), 41 samples (10.56%) using (BPAT), and 30 samples (7.73%) using (SAT) (Table I, Figure 2). The mRBPT yielded more positive results than the other two tests, with a statistically significant difference ($P < 0.0005$). All serum samples were subsequently retested using (cELISA) as confirmatory test, which confirmed that 32 samples (8.24%) were positive.

When comparing the screening tests to the confirmatory test, Cohen's kappa coefficients indicated poor agreement with cELISA: 16% for mRBPT, 32% for BPAT, and 31% for SAT. A kappa value of 40 or less is considered poor.

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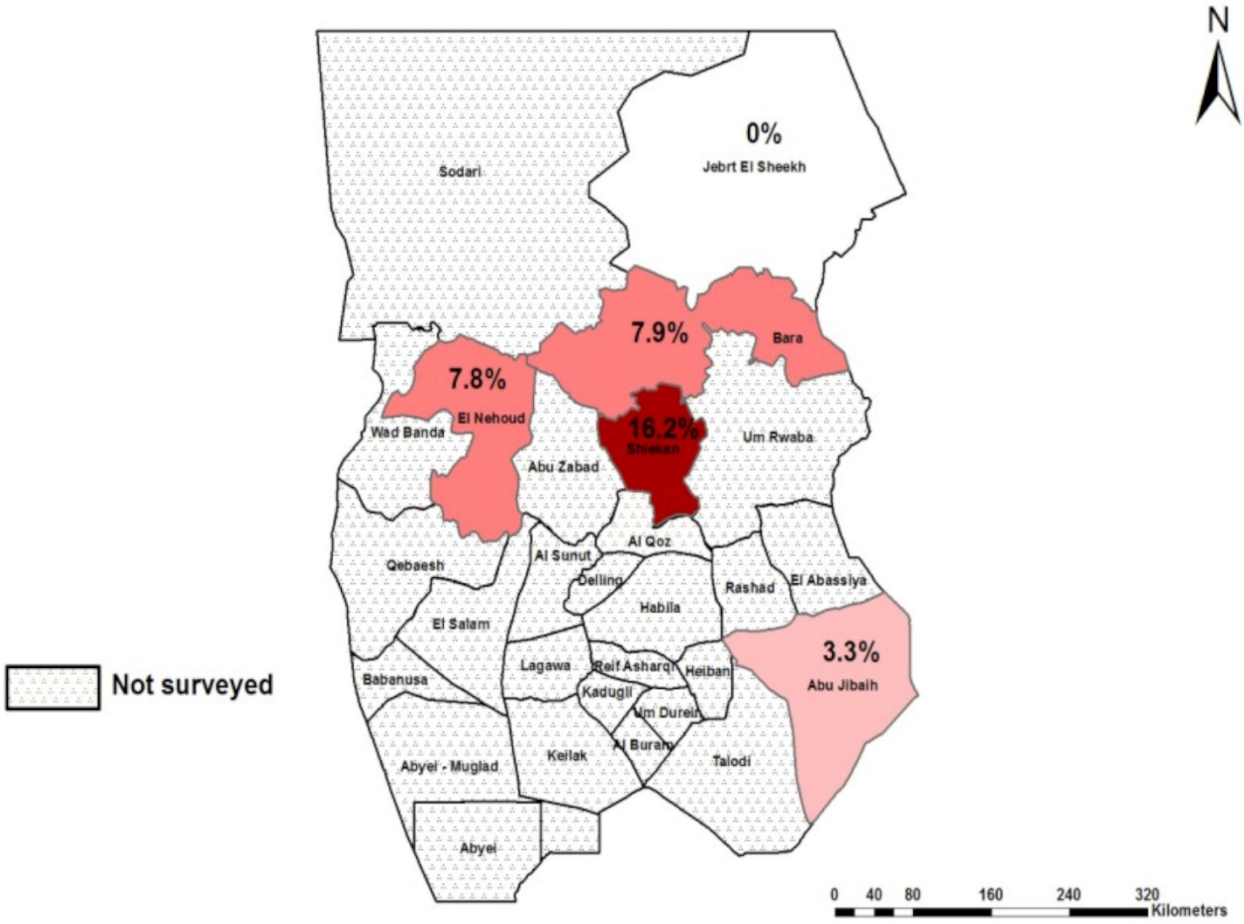

Figure 2. The prevalence of camel brucellosis in some localities in Kordofan States (North, West and South).

Results of Geographic Information System (GIS)

The point map illustrates that the distribution of brucellosis-positive camels is widespread throughout the central regions of Kordofan States (Fig. 2). The map identifies Shiekan locality in northern Kordofan State as having a high prevalence of 16.13%. In contrast, two localities in the northern and southern Kordofan States exhibited low prevalence rates, with Jebrt Elsheekh at 0% and Abu Jibiha at 3.3%.

Discussion

The spread of brucellosis in camels in a given area depends on the prevalence of *Brucella* species in other animals that share their habitat, as well as the husbandry methods employed. The mRBPT, BPAT, and SAT tests are known to be highly sensitive but not very specific, while the cELISA test is both specific and sensitive, effectively minimizing false positives by eliminating cross-reaction with other heterogeneous bacteria. Variations in prevalence between the mRBPT and SAT tests may be due to false positives caused by other bacteria that induce similar immunoglobulins.

The mRBPT test enhances the clarity of the test readings, as any visible agglutination is interpreted as a positive result. This observation supports the findings of Omer *et al*. (2010), who reported that mRBPT facilitated the reading of agglutination and recommended it for screening camel sera for brucellosis. In our investigation, 11.08%, 10.56%, and 7.73% of the camel sera were found positive by mRBPT, BPAT, and SAT, respectively. Notably, mRBPT detected more positive cases than the other two agglutination tests. This is consistent with earlier studies (Omer *et al*., 2010; Mohamed *et al*., 2015; Ahmed *et al*., 2017), which indicated that mRBPT is the most widely used test for screening brucellosis in camels. Conversely, there is limited literature on the application of cELISA to camel serum (Omer *et al*., 2007).

Our results showed that 32 out of 388 camel serum samples (8.24%) tested positive by cELISA, which is lower than the 87.5% reported by Mohamed *et al*. (2015) in Sudan and 68.8% reported by Gwida (2011) in camels from Egypt. cELISA has lower sensitivity compared to other serological tests, likely because it was specifically standardized for

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bovine sera. The Kappa coefficient agreement between cELISA and BPAT was 16%, while the agreement between cELISA and SAT was 31%, which reflects poor agreement (≤40% is considered poor).

The discrepancies in seroprevalence observed in this study compared to previous studies in Sudan and other regions of Africa can be attributed to several factors, including differences in the number of camels investigated, husbandry systems, the socioeconomic status of herders, and the sensitivity and specificity of the diagnostic methods used. Additionally, uncontrolled movement of animals and poor management practices may contribute to the transmission of brucellosis among camels, as noted by Musa *et al*. (2008).

The variations in prevalence across different locations are linked to pastoralist husbandry practices, which often involve camels sharing grazing areas and drinking from the same water sources, particularly in densely populated areas like Shiekan. These regions have a high concentration of camels due to abundant pastures and grazing land, increasing the risk of cross-transmission of the organism (Musa *et al*., 2008).

In this study, we employed Geographic Information System (GIS) technology to map the prevalence of the disease in various localities within Kordofan States. The purpose of mapping diseases is to evaluate the disease situation, identify areas where intervention is most likely to be technically, economically, socially, and environmentally sustainable, and inform the design of effective control and monitoring strategies against the disease.

Conclusion

This study's results indicate that brucellosis is present in camels from the Western States of Sudan. The findings, presented using Geographic Information Systems (GIS), can assist policymakers in planning health services and making informed decisions regarding the control of infectious diseases.

Acknowledgments

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Author Contribution

Maha I Khojaly and Enaam Mohamed El Sanousi: contributed to the conception and design of this study, performed the literature collection. Maha I Khojaly wrote the first draft of the manuscript. Suhaib Abdelrahman Salih: collection of samples, Selma.K .Ahmed: Statistical analysis of the result, Mohammed Taha Shigidi: Supervision. All authors have read and agreed to the published version of the manuscript.

Conflicts of interests

The authors declare no conflict of interest.

Ethical statement

Statement of animal rights: All procedures performed with animals in the present study were in accordance with the ethical standards established by the Ethical Committee of the central veterinary Research Laboratory (CVRL), Sudan.

References

Abbas, B.; Agab, H. (2002). A review of camel brucellosis. Preventive veterinary Medicine, 55:47-56.

Al-Juboori A, Baker MM. (2012). Studies on common reproductive disorders in dromedary Camels (Camelus dromedarius) in United Arab Emirates (UAE) under field conditions. Proceedings of the 3rd Conference of the International Society of Camelid Research and Development (ISOCARD). pp: 127-128. Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Muscat, Sultanate of

Oman.

Anon. (2011). Reports of the general directory of quarantines and meat hygiene, Ministry of Animal resources, fisheries and land ranges. Sudan.

Blasco, J.M., Garin-Bastuji, B; Marin, C.M;Gerbier, G; Fanlo; J, Jimenez de Bagues, M.P; Cau, C. (1994). Efficacy of different Rose Bengal and Complement Fixation antigens for the diagnosis of Brucella melitensis infection in sheep and goats. Vet. Rec. 134 (16):415-420.

Calfee, M. & Wendling, Morgan. (2012). The effects of environmental conditions on persistence and inactivation of Brucella suis on building material surfaces. Letters in applied microbiology. 54. 504-10. 10.1111/j.1472-765X.2012.03237.x.

Dawwod, A.H., (2008). Brucellosis in Camels (CAMELUS DROMEDARIUS) in the South Province of Jordan. American Journal of Agricultural and Biological Sciences 3(3):623-626.

Davies, G. and Casey, A. (1973). The survival of Brucella abortus in milk and milk products. Brit. Vet. J., 129: 345 - 353.

ElSanousi, Enaam M. (2006). Molecular Diagnosis of Brucella species. Ph.D. Thesis, University of Khartoum, Sudan.

FAO. 2010. Brucella melitensis in Eurasia and the Middle East. FAO Animal Production and Health Proceedings. No. 10. Rome.
FAOSTAT (2018). http://www.fao.org/faostat/en/#data/QA.

Gwida, M.M., El-Gohary, A. H., Melzer, F., Tomaso, H., Rösler, U., Wernery, U., Wernery, R., Elschner, M.C., Khan, L., Eickhoff, M., Schöner, D., Neubauer, H. (2011). Comparison of diagnostic tests for the detection of Brucella spp. in camel sera. BMC Res Notes. 6 (4): 525.

Gwida, M.; El-Gohary, A.; Melzer, F.; Khan, I.; Rösler, U. and Neubauer, H. (2012). Brucellosis in camels. Res. Vet. Sci., 92:351-355.

HalaElrayah, A. 1; Huda, A. O1; Yagoub, I. A2 and El Sanousi, Enaam, M (2015). Survey of Brucellosis in Camels Intended for Export in North Kordofan State, Western Sudan. The Regional Conference of Camel Management and Production under Open range System (RCCMPR), Khartoum-Sudan, and 2nd -4th March 2015.

Jacques, V. Olivier-Bernardina, G. Dubraya (1998): Efficacy of ELISA compared to Conventional tests (RBPT and CFT) for the diagnosis of Brucella melitensis infection in sheep. Veterinary Microbiology (64) pp 61-73.

Mahasin Mohammed Ahmed, Hatim Hamad Ibrahaem, Yassir Adam Shuaib, Ali Abdelgani Elgadal, Mohamed Taha Shigidi. (2017). Sero-Prevalence of Anti-Brucella Antibodies in Camels in El-Gedarif State: A Short Communication; Sch J Agric Vet Sci., Nov 2017; 4(11):461-463.

Musa, M. T., Eisa, M. Z. M., El Sanousi, E. M., Abdel Wahab, M. B. and Perrett, L., (2008). Brucellosis in Camels (Camelus dromedarius) in Darfur, Western Sudan. J. Comp. Path. 138:151-155.

Musa, M.T Shigidi (2001). Brucellosis in camels in intensive Animal breeding areas of Sudan. Implication in abortion and early life infection. Revue. Elev. Med, vet. Pajs trop, 54: 11-15. Omer MM, Abdelaziz AA, Abusalab MS, Ahmed MA: Survey of brucellosis among sheep, Goats, Camels and Cattle in Kassala Area, Eastern Sudan. JAnim Vet Adv 2007, 6:635-637.

Musa, T.M., Enaam, M.E., Angara, E.E. and Ali, A.A. (2008) Brucellosis, a Challenge to Veterinarians in Africa: The Situation of the Disease in the Sudan, the Proceedings of the First Scientific Conference (ARRC), Animal Resources Research Corporation, 17-21/8/2008. Khartoum, Sudan. P45-56.

Mohamed EGS, AAM Elfadi and EM El Sanousi. (2015).Epidemiological study of brucellosis in camels (Camelus dromedarius) in Khartoum State, Sudan. Inter J Vet Sci, 4(1): 39-43. www.ijvets.com.

Omer,M.M.; Musa, M.T.; Bakhiet, M. R. and Perrett, L. (2010a). Brucellosis in camels, cattle and humans: associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. Rev. Sci. Tech. 29: 663-669.

Thrusfield, M., (2005). Veterinary Epidemiology, Blackwell Publishing Company.

Wegdan, O.M., El-Fadil, A.A., Elgadal, A.A. and Shuaib, Y.A. (2016) Seroprevalence and risk factors of anti-brucella antibodies in cattle in Khartoum State, the Sudan. J. Adv. Vet. Anim. Res., 3: 134-144.

WOAH. (2022). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2022, Brucellosis (Infection with Brucella abortus, B. melitensis and B. suis). Chapter 3.1.4.B.2. Available online: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.04_BRUCELLOSIS.pdfs (accessed on 25 May 2022).

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