



Incidence and prevalence of tick-borne haemoparasites infecting sheep and goats in Sennar state, Sudan

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Abstract

Sudan possess more than ninety million head of sheep and goats. However, rare was reported regarding Babesiosis, Anaplasmosis and Theileriosis in sheep and goats. In the present study the prevalence of Babesia, Theileria and Anaplasma, the causative agents for sheep and goats piroplasmosis were investigated in Sennar state, Sudan, through a crosssectional study carried out based on Giemsa staining assay from November 2014 to October 2015. The study was performed in five locations namely Sennar, Singa, El Mazmum, Abu Na'ama and El Dinder. Of 1415 sheep and goats examined, 31.66% of the Sheep and 18.62% of the goats were positive for different Piroplasma species. Apparently, sheep had higher rate of Piroplasma species infection compared with goats. Additionally, in both animals the overall prevalence was higher in Babesia species followed by Theileria spp and then Anaplasma spp. Risk factor analysis showed that location and season were associated to sheep and goats piroplasmosis. Such results were discussed accordingly and recommendations were suggested to undertake the socio-economic importance, prevention and control of such diseases.

Keywords: sheep, goats, piroplasma species, sennar state, Sudan

Introduction

Sudan possess around 140 million livestock population. The Ministry of Animal Wealth estimates the sheep and goats population at 48.0 and 42.0 million heads, respectively [1]. Such asset is endangered by different endemic diseases, which collectively causes significant economic losses to the pastoral community. In Sudan unpredictable weather, sometime drought and scarcity of fodders oblige the pastoralists to move with their animals. However, diseased animals normally have less ability for movement, less feeding ability, less feed conversion ratio and hence, light weight less reproduction and more susceptibility for diseases infection. Additionally, such diseases impose economic losses by causing morbidity and mortality and harming the internal and international livestock trade. Furthermore, the management of such diseases also augment the cost. Trying to avoid such costs, light or no healthcare practices could result in endemicity of such diseases and the diseased animals become reservoir and ultimately leads to more incidences [2, 3]. Moreover, livestock and humans emerging and re-emerging zoonotic diseases are nowadays spread tremendously and has potential of crossing continental boundaries. In Sudan, detection of emerging diseases like Crimean-Congo hemorrhagic fever, a tick-borne virus, was also reported as a result of eating raw sheep meet [4]. The tick-borne diseases cause important economic losses particularly in developing countries [5]. Babesiosis, Anaplasmosis and Theileriosis are among the most important tick-transmitted diseases affecting animals in sub-Saharan Africa [6, 7]. In fact, literature survey revealed few reports about such diseases in sheep and goats in Sudan. Tick vectors

are different throughout the worldwide. For instance, *Rhipicephalus evertsi* and *Rhipicephalus (Boophilus) decoloratus* were reported as competent Babesiosis tick vectors in livestock in Angola [8]. However, in Sudan, such parasites were reported in small ruminants without relations as vectors to such tick species [9-16]. Therefore, the relation of such diseases to vectors is not understood. Additionally, some other factors might play important roles in such circulation of infection. For instance, climatic stress (e.g. fluctuation of raining) and hence, less weeds that leads to movement of animals in addition to possibility of acquiring other infection with other diseases such as trypanosomosis will lead to malnutrition beside the inadequate and poor veterinary service. Furthermore, the favorability of the such climate for tick survival and the availability of ticks on such hosts and lack of vaccination [17]. As a consequence, the basic information about current epidemiological status of such diseases in livestock is lacking. Therefore, as a contribution to increase the knowledge on presence and distribution of such diseases and vectors, the present work is considered as a step in a frame of broader and precise developmental research project.

Materials and Methods

Study area and Study animals

Sennar state is a plane area bordered by Gezira state in the north, Gadaref state in the east, Blue Nile state in the west and Ethiopian international border in the south. These included Sennar town (13°33' N, 33°37' E), Singa (13°09' N, 33°57' E), Dinder (13°44' N, 34°12' E), and Abu Naama (12°44' N,

34°08' E). Sheep and goats blood specimens were collected from around 5 cities: Sennar, Singa, Muzmum, Abu Na'ama and Dindir (Map 1).

Blood Samples Collection and Estimation of Parasitemias

Blood samples were collected from 717 sheep and 698 goats. For blood samples collection, around fifty animals (25 Watish sheep and 25 Nubian goats), were randomly selected from each of the aforementioned 5 locations every 2 month, year round from November 2014 to October 2015 during three seasons: Winter (November to February), Summer (March to June) and Autumn (July to October). Blood smears were prepared from the sheep and goats' jugular vein blood and examined using Giemsa method and observed at 1000× magnification as described [18, 19].

Statistical Analysis

Descriptive statistics using software STATISTIX-8 was used.

Results and Discussion

In Sudan, few PCR-based techniques were reported for research purpose, however, such techniques are not yet been documented for field application to diagnose small ruminant piroplasmosis [16]. Additionally, for limited budget, in the current work, only blood smear examination was used. Such blood smear diagnostic observations were considered therefore, as a step to be followed by molecular techniques reassessments that would provide more informative diagnosis. Bearing in mind the microscopy was the gold standard diagnosis method used in the present study, such a high prevalence encourages scientists to be confirmed by most sensitive methods in both animals' blood and tick species to explained the biological transmission. Additionally, among the tick species found in the present study (in process for publication), some were reported elsewhere as competent vector ticks for bovine babesiosis such as *Rhipicephalus evertsi*, *R. sanguineus* and *R. (Boophilus) decoloratus* [8, 20, 21]. Furthermore, Theileriosis is a disease caused by species of the genus *Theileria*, and transmitted by tick genera such as

Rhipicephalus [22]. Moreover, it worth noting that such diseases prevalence could also be due to mechanical transmission by biting flies and also by vertical transmission [23].

Of 1415 sheep and goats examined, 31.66% of the Sheep and 18.62% of the goats were positive for different Piroplasma species (Table 1). Apparently, sheep had higher rate of Piroplasma species infection compared with goats. Additionally, in both animals the overall prevalence was higher in Babesia species followed by Therileria spp and then Anaplasma spp. Risk factor analysis showed that location and season were associated to sheep and goats piroplasmosis. The present results provide important information that could be beneficial for better understanding, prevention and control of such diseases.

Table (2) shows the percentages of Babesia, Anaplasma and Therileria [total 227 infected sheep out of 717 examined animal (31.66%)] in sheep blood year-round (3 seasons) in Sennar state. The positives were breakdown as: Babesia spp. 106 infected sheep (46.70%) predominated followed by Theileria spp 76(33.48%) and then, Anaplasma spp 45(19.82%). Regarding the effect of location in Babesia infection, the prevalence percentage is in descending order as follows: Sennar, Singa, Mazmum, Dindir and Abu Na'ama. With regard to Theileria, it was higher in Singa, followed by Dindir, Mazmum, Sennar and then, Abu Na'ama. However, the Anaplasma prevalence percentage was in descending order as: Singa, Dindir, Mazmum, Abu Na'ama and then, Sennar. The higher prevalence was in winter, then summer and autumn.

Table 1: Total percentage prevalence of the different *Piroplasma* spp. in sheep and goats in Sennar state, Sudan from November 2014 to October 2015

	Babesia	Theileria	Anaplasma	Total
Sheep	106	76	45	227
(717)	46.70%	33.48%	19.82%	31.66%
Goats	79	37	14	130
(698)	60.77%	28.46%	10.77%	18.62%

Table 2: Effect of location and season on the prevalence of Babesia, Therileria and Anaplasma spp. in sheep blood at Sennar state, Sudan November 2014 to October 2015

Location	Babesia spp.			Anaplasma spp.			Theileria spp.		
	Winter 1	Summer	Autumn	Winter	Summer	Autumn	Winter	Summer	Autumn
Sennar	30.4	12.0	10.2	2.2	0.0	0.0	6.5	14.0	10.2
Singa	14.9	22.0	12.2	4.3	0.0	4.1	14.9	10.0	12.2
Mazmum	15.7	24.1	6.3	35.3	7.4	2.1	5.9	20.4	6.3
Abu Na'ama	8.3	4.1	19.2	16.7	4.1	5.8	4.2	8.2	7.7
Dindir	14.6	11.8	16.0	6.3	2.0	0.0	12.5	17.6	4.0

As in sheep, in goats (Table 3), 130 goats out of 698 (18.62%) were found infected. Babesia spp was most predominated 79(60.77%) followed by Theileria spp 37(28.46%) and then Anaplasma spp 14(10.77%). Babesia proportional distribution was in descending order as follows: Singa, Sennar, Abu Na'ama, Dindir and then Mazmum. While Babesia spp seasonal distribution in winter and autumn was more higher than summer. However, the prevalence of Theileria spp was higher in Singa, less in Abu Na'ama, then, Sennar and no infection was detected in both Mazmum and Dindir. The

Theileria spp seasonal distribution seems as a slight normal distribution curve: winter raised up in summer and descended in autumn. Regarding the Anaplasma, the lesser parasites of the three in both sheep and goats. It was most predominated in Singa followed by Mazmum, Dindir, Sennar and non in Abu Na'ama. The Anaplasma spp seasonal prevalence was higher in winter then, summer and far less in autumn. Apparently, such diseases cause serious economic losses due to mortality and reduction of meat and milk yield. Moreover, the international trade and global animal enterprise would be

menaced [24-26]. Unfortunately, few research among Sudanese pastoralists or agro-pastoralists to challenge such problems was performed [27]. In Sudan, piroplasmosis epidemiological aspects are rarely reported in sheep [12, 28] and, goats [29]. Using Reverse Line Blot (RLB) hybridization assay, it was examined apparently healthy sheep (n=219) from six different geographical localities in Sudan [16]. They scored *Theileria ovis*, *T. separata*, *T. lestoquardi* and *T. annulata* and stated that *T. ovis* and *T. separata* were reported for the first time in

sheep in Sudan. However, there is no *Babesia* species detected in all examined samples and that could be due to the use of 15 oligonucleotide probes that could be unspecific for *Babesia* species. Moreover, such prevalence of *Anaplasma* in sheep and goat herds in Sudan as part of the sub-Saharan Africa is not a surprise [30]. Additionally, despite the presence of the three aforementioned blood parasites, prevalence and genetic diversity of *Babesia* and *Anaplasma* species in cattle in Sudan was reported [31].

Table 3: Effect of location and season on the prevalence of *Babesia*, *Theileria* and *Anaplasma* spp. in goats' blood at Sennar State, Sudan November 2014 to October 2015

Location	Babesia spp.			Anaplasma spp.			Theileria spp.		
	Winter	Summer	Autumn	Winter	Summer	Autumn	Winter	Summer	Autumn
Sennar	19.1	13.6	3.9	0	2.3	0	8.5	9.1	3.9
Singa	14.3	13.7	34.6	4.1	0	14	14.3	11.8	1.9
Mazmum	8.9	0	4.1	2.2	0	2	0	0	0
Abu Na'ama	15.9	6.3	10.4	0	0	0	0	10.4	16.7
Dindir	9.8	6	4	0	4	0	0	0	0

In fact, in the present project, ten tick species were found in sheep and goats (data in process for publication). It was reported that the immature stages of tick can obtain the disease by feeding on the infected animal and transmit it transstadially. Additionally, the infected female adult tick can spread such diseases up to 32 generations via a transovarial route [32]. Furthermore, such parasites new genotypes were reported [33]. It is therefore, would be valuable should an elaborative study on the genetic diversity of such parasites and routes of transmission in Sudan is considered to fill the gap of such an overlooked areas. Generally, for better understanding of the ecology of a certain disease in a certain geographical region, in a certain animal species and animal breed, precise evaluation should be planned regarding the parasites and the vectors. Therefore, further study including precise parasites diagnosis methods in both animal serum and ticks beside close monitoring of distribution of tick vectors should be carried out to clarify present and recent epidemiological threats, estimate the most imperiled areas by such haemoparasites and best management program to reduce the economic losses in Sudan. Furthermore, such tropical diseases are one of the major hurdles of animal production. There are several risk factor lead to the occurrence and augmenting the harm of such diseases. For instance, climate changes and raining, expansion of cultivating land, deforestation in benefit of farming, effect on vectors, lack of public awareness, the close contact between livestock and owners and labors and emergence of new pathogens' serotype that facilitate them to escape from present control measures. That in addition to the effect of the indigenous animal breeds vs. crossed ones and the intensity of the cross-breeding programs and quality of vaccination [34, 35]. It is therefore a fundamental requirement to establish a synchronized monitoring and surveillance of such diseases, including geographical information system (GIS) [36] and rapid and confirmatory diagnosis throughout the country. Additionally, because Sennar state borders Ethiopia, such steps would be crucial for the management, control and eradication of endemic and trans-boundary diseases of livestock. Such measures lead to improve quality and quantity

of livestock products hence, compete in the international market [37]. Therefore, the involvement of the political decision makers would be crucial for avoiding hiding of the disease outbreaks, quarantine of diseased animals and implementation of national and regional collaboration, transport of clinical materials for diagnosis and vaccines. The aforementioned efforts could help providing effective management and therefore, sustainable livestock production.

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References

1. FAO/Sudan. Rapid assessment report: Potential impacts of El Nino on livestock in Sudan, November 23, 2015, Khartoum, Sudan.
2. Chand K, Biswas SK, Pandey AB, Muthuchelvan D, Mondal B. Bluetongue in India: A review. *Advances in Animal and Veterinary Sciences*, 2015; 3:605-612.
3. Kumar R, Singh SP, Savalia CV. Overview of Emerging Zoonoses in India: Areas of Concern. *Journal of Tropical Diseases*. 2015a; 3:165.
4. Aradaib IE, Erickson BR, Karsany MS, Khristova ML, Elageb RM, Mohamed MEH, *et al.* Multiple Crimean-Congo hemorrhagic fever virus strains are associated with disease outbreaks in Sudan, 2008-2009. *PLoS Negl Trop Dis*. 2011; 5(5):e1159. doi: 10.1371/journal.pntd.0001159.
5. Uilenberg G. International collaborative research:

- significance of tick-borne haemoparasitic diseases to world animal health. *Veterinary Parasitology*. 1995; 57:19-41.
6. Yusufmia SBAS, Collins NE, Nkuna R, Troskie M, Van den Bossche P, Penzhorn BL. Occurrence of *Theileria parva* and other haemoprotozoa in cattle at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. *Journal of South African Veterinary Association*. 2010; 81:45-49.
 7. Gachohi JM, Ngumi PN, Kitala PM, Skilton RA. Estimating seroprevalence and variation to four tick-borne infections and determination of associated risk factors in cattle under traditional mixed farming system in Mbeere District, Kenya. *Preventive Veterinary Medicine*. 2010; 95:208-223.
 8. Gomes AF, Pombal Jr AM, Venturi L. Observations on cattle ticks in Huila Province (Angola). *Veterinary Parasitology*. 1994; 51:333-336.
 9. Jongejan F, Zivkovic D, Pegram RG, Tatchell RJ, Fison T, Latif AA, *et al.* Ticks (Acari: Ixodidae) of the Blue and White Nile ecosystems in the Sudan with particular reference to the Rhipicephalus sanguineus group. *Experimental and Applied Acarology*. 1987; 3:331-346.
 10. El Imam AH. Ecological studies on ticks infesting cattle Kosti, Sudan. *Sudan Journal of Veterinary Science and Animal Husbandry*. 2003; 42:62-71.
 11. El Ghali AA, Hassan SM. Ticks infesting animals in the Sudan and Southern Sudan: Past and current status. *Onderstepoort Journal of Veterinary Research*. 2012; 79:431-436.
 12. Salih DA, El Hussein AM, Hayat MF, Taha KM. Survey of *Theileria lestoquardi* antibodies among Sudanese sheep. *Veterinary Parasitology*. 2003; 111:361-367.
 13. Hassan SM, Salih DA. An overview of factors responsible for geographic distribution pattern of ixodid ticks in the Sudan. *Sokoto Journal of Veterinary Sciences*. 2013; 11:1-9.
 14. El Imam AH, Taha KM. Malignant ovine theileriosis (*Theileria lestoquardi*): a review. *Jordan Journal of Biological Sciences*. 2015; 8:165-174.
 15. El Imam AH, Hassan SM, Gameel AA, El Hussein AM, Taha KM, Salih DA. Variation in susceptibility of three Sudanese sheep ecotypes to natural infection with *Theileria lestoquardi*. *Small Ruminant Research*. 2015; 124:105-111.
 16. El Imam AH, Hassan SM, Gameel AA, El Hussein AM, Taha KM, Oosthuizen MC. Molecular identification of different *Theileria* and *Babesia* species infecting sheep in Sudan. *Annals of Parasitology*. 2016; 62(1):47-54. doi: 10.17420/ap6201.31
 17. Kumar V, Kaur P, Wadhawan VM, Pal H, Sharma H, Kumar P. Theileriosis in cattle: prevalence and seasonal incidence in Jalandhar district of Punjab (India). *International Journal of Recent Scientific Research*. 2015; 6:2998-2999.
 18. Bose R, Jorgensen WK, Dalgliesh RJ, Friedhoff KT, de Vos AJ. Current state and future trends in the diagnosis of babesiosis. *Vet Parasitolol*. 1995; 57(1-3):61-74.
 19. Kemal J. Laboratory Manual and Review on Clinical Pathology, Published by OMICS Group eBooks, 731 Gull Ave, Foster City. CA 94404, USA, 2014.
 20. Bock R, Jackson L, De Vos A, Jorgensen W. Babesiosis of cattle. *Parasitology*. 2004; 129:S247-S269.
 21. Kocan KM, de La Fuente J, Blouin EF, Garcia-Garcia JC. *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. *Parasitology*. 2004; 129:S285-S300.
 22. Olwoch JM, Reyers B, Engelbrecht FAB, Erasmus FN. Climate change and the tick-borne disease, theileriosis (East Coast fever) in sub-Saharan Africa. *Journal of Arid Environments*. 2008; 72:108-120.
 23. De Waal DT. Anaplasmosis control and diagnosis in South Africa. *Annals of the New York Academy of Sciences*. 2000; 916:474-483.
 24. Rapoport E, Shimshony A. Health hazards to the small ruminant population of the Middle East posed by the trade of sheep and goat meat. *Rev Sci. Tech*. 1997; 16(1):57-64.
 25. Sherman DM. The spread of pathogens through trade in small ruminants and their products. *Rev Sci. Tech*. 2011; 30(1):207-217.
 26. Depa PM, Dimri U, Sharma MC, Tiwari R. Update on epidemiology and control of Foot and Mouth Disease - A menace to international trade and global animal enterprise. *Veterinary World*. 2012; 5:694-704.
 27. Casciarri B, Ahmed AGM. Pastoralism under pressure in present-day Sudan: an introduction. *Nomadic Peoples*. 2009; 13:10-22.
 28. Taha KM, Salih DA, Ali AM, Omer RA, El Hussein AM. Naturally occurring infections of cattle with *Theileria lestoquardi* and sheep with *Theileria annulata* in the Sudan. *Veterinary Parasitology*. 2013; 191:143-145.
 29. Taha KM, El Hussein AM, Abdalla HS, Salih DA. *Theileria lestoquardi* infection in goats in River Nile State: comparison of serology and blood smears. *Sudan Journal of Veterinary Science and Animal Husbandry*. 2003; 42:197-206.
 30. Bell-Sakyi L, Koney EBM, Dogbey O, Walker AR. Incidence and prevalence of tick-borne haemoparasites in domestic ruminants in Ghana. *Veterinary Parasitology*. 2004; 124:25-42.
 31. Awad H, Antunes S, Galindo RC, do Rosário VE, de la Fuente J, Domingos A, *et al.* Prevalence and genetic diversity of *Babesia* and *Anaplasma* species in cattle in Sudan. *Vet Parasitol*. 2011; 181(2-4):146-52. doi: 10.1016/j.vetpar.2011.04.007. Epub 2011 Apr 12.
 32. Saminathan M, Rajneesh R, Ramakrishnan MA, Karthik K, Malik YS, Dhama K. Prevalence, diagnosis, management and control of important diseases of ruminants with special reference to Indian scenario. *Journal of Experimental Biology and Agricultural Sciences*. 2016; 4(3S):338-367. DOI: [http://dx.doi.org/10.18006/2016.4\(3S\).338.367](http://dx.doi.org/10.18006/2016.4(3S).338.367)
 33. George N, Bhandari V, Reddy DP, Sharma P. Molecular and Phylogenetic analysis revealed new genotypes of *Theileria annulata* parasites from India. *Parasites and Vectors*. 2015; 8:468.

34. Dhama K, Wani MY, Tiwari R, Kumar D. Molecular diagnosis of animal diseases: the current trends and perspectives. *Livestock Sphere*, 2012, 6-10.
35. Kohli S, Atheya UK, Srivastava SK, Banerjee PS, Garg R. Outbreak of theileriosis and anaplasmosis in herd of Holstein crossbred cows of Dehradun district of Uttranchal, India: A Himalyan region. *International Journal of Livestock Production*. 2014; 5:182-185.
36. Dhama K, Verma AK, Tiwari R, Chakraborty S, Vora K, Kapoor SR Deb, *et al.* A perspective on applications of geographical information system (GIS); an advanced tracking tool for disease surveillance and monitoring in veterinary epidemiology. *Advances in Animal and Veterinary Sciences*. 2013; 1:14-24.
37. Awase M, Gangwar LS, Patil AK, Goyal G, Omprakash. Assessment of economic losses due to Peste des Petits Ruminants (PPR) disease in goats in Indore Division of Madhya Pradesh. *Livestock Research International*. 2013; 1:61-63.