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Detection of Theileria and Babesia infection and their co-infection in ruminants in Wad Madani, Gezira State, Sudan

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Abstract : *The Poor animal health is often a consequence of parasitic diseases; ticks are transmitting a number of diseases to indigenous and exotic ruminants in Sudan and cause large economic losses in productivity. This study was designed to investigate Theileria and Babesia infection in ruminants (cows, sheep and goats) from ruminant's farms and ruminants market (Al mwashi market) in Wad Madani town, central Sudan. A total of 130 blood samples were collected from December 2020 to March 2021. Samples were collected from ruminants from 13 farms in Wad Madani town and Al mwashi market. Ruminants were chosen according to the breed, sex and age. For hematological examination for identification of the parasites in the prepared blood smears which were tested using Giemsa stain. Then Complete Blood Count (CBC) were done for each ruminants, measuring RBC, WBC, LYM, MID, GRAN, MCH, MCHC, MCV, HGB, HCT and RDW. The result revealed that 30 (23.1%) samples were positive for co-infection of Babesia and Theilera. (22.5%) in cows, (15.4%) in goats and (29.4%) in sheep. Results showed that sheep are the most one which have the co-infection with both Theileria and Babesia.*

Keywords: Theileria, Babesia, Sudan.

Introduction:

Tick-borne diseases (TBDs) are present throughout the world especially in the tropical and subtropical regions and affect approximately 80% of the world's ruminants population. They are considered a significant threat to the global food security (Ibrahim *et al.*, 2020). Losses directly attributed to haemoparasitic diseases include morbidity, mortality, production losses together with the costs of veterinary diagnosis/treatment and vector control (Ganguly *et al.*, 2020). The economically most important tick-borne diseases are theileriosis, babesiosis, ehrlichiosis (heart water) and anaplasmosis (ElGhali and Hassan, 2012). Tropical theileriosis is a parasitic disease caused by the hemoprotozoan *Theileria annulata* that is transmitted to ruminants by ixodid ticks of genus Hyalomma (Uilenberg *et al.*, 2013). *H. anatolicum* is the most important tick species in ruminants and buffalo (Hayati *et al.*, 2020). *Theileria annulata* is widely distributed throughout Europe, the Middle East, Russia, China, and Africa, whereas *Theileria parva*, termed East Coast fever, is primarily distributed in Africa (Jia, *et al.*, 2020). It is a destructive disease that affects ruminants of all ages, breeds and sex and leads to severe losses in production and reproduction. Tropical theileriosis is highly endemic in Al Gezira State, Central Sudan due to the high prevalence of *H. anatolicum*, which is the main vector of this disease (Ibrahim *et al*, 2020). Clinical signs shown by the affected animal include high fever (107^{O}F), anorexia, weight loss, debility, long lasting anaemia, jaundice, tachypnea, cough, salivation, lacrimation, petechiae on conjunctiva, enlarged lymph nodes and abortions or stillbirths (Reddy and Lakshman, 2020). Nasal discharge, enlargement of superficial lymph nodes, anorexia, reduced appetite (Niaz *et al.*, 2021). Lethargy, increased cardiac and respiratory rates, respiratory failure, decreased milk production (Eamens, *et al.*, 2013; Khatoon *et al.*, 2015). Ruminants that are left untreated usually die from the disease (Anupama *et al.*, 2015). Beside to that Bovine babesiosis is a tick-borne (Tb) parasitic disease of ruminants caused by *Babesia* species, a haemoprotozoan parasite including two predominant species i.e. *B. bigemina* and *B. bovis*. These species mostly affect ruminants and buffalos and are prevalent worldwide. With significant distribution in the tropical and subtropical areas of Africa, Asia, Australia, central and South America (Siddique *et al.*, 2020). The transmission of the disease by ticks and the principal of vectors of *B. bovis and B. bigemina are Rhipicephlaus* formally *Boophilus* species (Embiyale *et al.*, 2018). *Babesia divergens*, transmitted by the tick *Ixodes ricinus* (Springer *et al.*, 2020). Affected animals suffered from marked rise in body temperature, loss of appetite, cessation of rumination, labored breathing, emaciation, progressive hemolytic anemia, various degrees of jaundice (Icterus) (Yusuf, 2017). From paleness in mild case to sever yellow discoloration of conjunctival and vaginal mucous membranes in more progressive cases; haemoglobinuria, accelerated heart and respiratory rates, ocular problems and drop in milk production. In ruminants, fever during infections in some cases cause abortion to pregnant ruminants (El Moghazy *et al.*, 2014). Coffee colored urine is the characteristics clinical feature of babesiosis (Wodaje *et al.*, 2019). The detection methods of *Babesia* and *Theileria* spp. are morphological examination (Habibi et *al.*, 2020). Smears are preparations made using liquid substances. The function of making smears preparations is to observe cells in body fluids, for example

in the blood. Diagnostic techniques were tested using Giemsa's staining technique. The blood parasites were then identified and their prevalence was determined (Ritonga *et al.,* 2020).

Materials and methods

Study design area and population:

Cross sectional study for ruminants, conducted during the period of December 2020 – June 2021 was take place at Wad Madani town, Gezira State, Central Sudan. Which it is located 5,223.49 mi (8,406.40 km) south of the North Pole and has a hot desert climate. Two sites were chosen according to the availability of animals: 13 private ruminant's farms and the main ruminants market in the town (Al mwashi market). Ruminants were chosen according to the breed, sex and age. The age ranged between >1 - <3 years, mostly sheep and goats were examined, followed by cows. The number of males (25, 13, 15) and the females were (26, 26, 25) animals respectively.

Three species of ruminants were chosen to investigate Theileria and Babesia infection (cows, sheep and goats). A total of 130 blood samples were collected: 40 from cows, 39 from sheep and 51 from goats. Sample size was determined according to the availability of the animals and willing of the owners to include their animals in the study.

Blood collection:

Three ml of blood collected by disposable sterile syringes from each animal in heparized tubes for Complete Blood Count (CBC), thin and thick blood smears stained with Giemsa stain were done.

Complete Blood Cell Count (CBC):

Complete Blood Count (CBC) were done from the whole blood of each ruminant for analysis of: RBC, WBC, LYM, MID, GRAN, MCH, MCHC, MCV, HGB, HCT and RDW.

Blood Smear Technique (BST):

Thin blood smears were prepared from each sample. The blood smears were made in newly labeled glass slides according to McCosker(1975) by putting ne drop of blood on a microscope slide and using another slid to spread the blood at an acute angle that makes a thin film. Then slides were air died and fixed by absolute methanol for 2-3 minutes before staining using 10% solution of Giema stain for 30 minutes. The slides were washed with distilled water; air dried. Stained thin blood films examined by microscope using the X100 magnification using oil immersion lens for presence of *Theileria* and *Babesia spp*. piroplasms.

Results:

Infection rate according to breeds:

Results showed **Babesia** infection rate was highest in sheep (51.0%), and then in cows (30.0%) and the lowest infection rate was in goats (20.5%). The **Theileria** infection rate was highest in sheep (64.7%), and then in goats (56.4%) and the lowest infection rate was in cows (55.0%). While the percentage of the **co-infection** with **Babesiosis** and **Theileriosis** was highest in sheep (29.4%), and then in cows (22.5%) and the lowest infection rate was in goats (15.4%) Table (1).

Table (1): Infection rate according to breeds:

Breed	Babesiosis (%)	Theileriosis (%)	Co-infection (%)	Total
Cows	12 (30.0)	22 (55.0)	9 (22.5)	40
Goats	8 (20.5)	22 (56.4)	6 (15.4)	39
sheep	26 (51.0)	33 (64.7)	15 (29.4)	51
Total	**46 (35.4)**	**77 (59.2)**	**30 (23.1)**	**130**

Infection rate according to age:

Babesia infection rate according to age group was highest in cows at the age group 1-3 years (35.3%), and then in cows less than 1 year (33.3%) and the lowest infection rate was in cows more than 3 years (18.2%).

Babesia infection rate according to age group was highest in goats less than 1 year (36.8%) and then in goats more than 3 years (14.3%), while the lowest infection rate was in goats between 1-3 years (0%).

Babesia infection rate according to age group was highest in sheep more than 3 years (66.7%) and than in sheep between 1-3 years (52.6%), while the lowest infection rate was in sheep less than 1 year (40%).

Theileriosis infection rate according to age group was highest in cows at age group 1-3 years (70.6%) and then cows less than 1 year (50%) while the lowest infection rate was in cows more than 3 years (36.4%).

Theileriosis infection rate according to age group was highest in goats at age group less than 1 year (68.4%) and then in goats between 1- 3 years (53.8%), while the lowest infection rate was in goats more than 3 years (28.6%).

Theileriosis infection rate according to age group was highest in sheep at age group more than 3 years (66.7%) and then in sheep less than one year (65%), while the lowest infection rate was in sheep between 1-3 years (63.2%).

Co-infection infection rate according to **age** in cows was highest at age group less than one year (33.3%) and then in cows more than 3 year (18.2%), while the lowest infection rate was in cows between 1- 3 years (17.6%).

Co-infection infection rate according to **age** in goats was highest at age group less than 1 year (13.6%) and then in the other categories (0%).

Co-infection infection rate according to **age** in sheep was highest at age group more than 3 years (41.7%) and then in sheep between 1-3 years (31.6%) while the lowest infection rate was in sheep less than 1 year (20%), Table (2).

Table (2): Infection rate according to age

Breed	Age group	Babesiosis (%)	Theileriosis (%)	Co-infection (%)	Total
Cows	<1 year	4 (33.3)	6 (50)	4 (33.3)	**12**
	1-3 years	6 (35.3)	12 (70.6)	3 (17.6)	**17**
	> 3 years	2 (18.2)	4 (36.4)	2 (18.2)	**11**
Goats	<1 year	7 (36.8)	13 (68.4)	6 (13.6)	**19**
	1-3 years	0 (0)	7 (53.8)	0 (0)	**13**
	> 3 years	1 (14.3)	2 (28.6)	0 (0)	**7**
Sheep	<1 year	8 (40)	13 (65)	4 (20)	**20**
	1-3 years	10 (52.6)	12 (63.2)	6 (31.6)	**19**
	> 3 years	8 (66.7)	8 (66.7)	5 (41.7)	**12**

Infection rate according to sex:

Babesia infection rate according to **sex** in cows was higher in males (40%) than in females (24%).
Babesia infection rate according to **sex** in goats was higher in males (30.8%) than females (15.4%).
Babesia infection rate according to **sex** in sheep was higher in males (52%) than females (50%), Table (3).
Theileria infection rate according to **sex** in cows was higher in females (60%) than males (46.7%).
Theileria infection rate according to **sex** in goats was higher in males (61.5%) than females (53.8%).
Theileria infection rate according to **sex** in sheep was higher in females (69.2%) than males (60%).
Co-infection infection rate according to **sex** in cows was higher in females (24%) than males (20%).
Co-infection infection rate according to **sex** in goats was higher in males (23.1%) than females (11.5%).
Co-infection infection rate according to **sex** in sheep was higher in females (34.6%) than males (24%) Table (3).

Table (3): Infection rate according to sex

Breed	Sex	Babesiosis (%)	Theileriosis (%)	Co-infection (%)	Total
Cows	Male	6 (40)	7 (46.7)	3 (20)	15
	Female	6 (24)	15 (60)	6 (24)	25
Goats	Male	4 (30.8)	8 (61.5)	3 (23.1)	13
	Female	4 (15.4)	14 (53.8)	3 (11.5)	26
Sheep	Male	13 (52)	15 (60)	6 (24)	25
	Female	13 (50)	18 (69.2)	9 (34.6)	26

Association between infection rate and CBC parameters:

Small ruminants (goats and sheep)

Results of infections (babesia, theileria and their co-infection) and CBC parameters indicated that there were significant association only between babesia infections and some CBC parameters (LYM% (0.022), GRAN% (0.018), MCH (0.006) and MCHC (0.008)). Table (4).

Table (4): Association between infection rate and CBC parameters in small ruminants (goats and sheep)

CBC	Babesiosis -ve (N56)	Babesiosis +ve (N34)	Theileriosis -ve (N35)	Theileriosis +ve (N55)	Co-infection -ve (N69)	Co-infection +ve (N21)
WBC 10^3/ul	34.51	38.56	34.61	36.19	36.47	34.62
***p*-value**	0.299		0.686		0.679	
LYM%	50.74	60.29	53.65	54.79	53.31	57.77
***p*-value**	**0.022***		0.787		0.358	
MID%	10.65	8.83	9.59	10.19	10.05	9.66
***p*-value**	0.148		0.645		0.788	
GRAN%	38.61	30.91	36.75	35.03	36.64	32.61
***p*-value**	**0.018***		0.606		0.220	
RBC10^6/ul	2.43	3.06	2.48	2.70	2.58	2.96
***p*-value**	0.097		0.552		0.367	
HGB g/dl	8.65	8.68	8.58	8.57	8.78	8.24
***p*-value**	0.950		0.977		0.341	
HCT%	10.87	13.73	11.05	12.14	11.53	13.32
***p*-value**	0.108		0.529		0.377	
MCV fl	43.56	44.16	43.20	43.38	43.70	44.05
***p*-value**	0.200		0.673		0.531	
MCH pg	62.20	39.69	55.34	51.93	57.60	40.85
***p*-value**	**0.006***		0.683		0.050	
MCHC g/dl	150.04	93.49	132.85	125.82	138.35	96.89
***p*-value**	**0.008***		0.742		0.059	
RDW-SD fl	17.24	17.20	17.29	17.18	17.22	17.23
***p*-value**	0.890		0.698		0.973	
RDW-CV%	12.37	12.17	12.38	12.25	12.32	12.22
***p*-value**	0.266		0.469		0.611	

Cows:

Results of infections (babesia, theileria and their co-infection) and CBC parameters indicated that there were significant association only between babesia infections and some CBC parameters (MCH (0.003) and MCHC (0.003)). Table (5).

Table (5): Association between infection rate and CBC parameters in cows

CBC	Babesiosis Negative (N28)	Babesiosis Positive (N12)	Theileriosis Negative (N18)	Theileriosis Positive (N22)	Co-infection Negative (N31)	Co-infection Positive (N9)
WBC 10^3/ul	27.04	25.92	27.62	26.70	27.57	25.54
***p*-value**	0.727		0.759		0.447	
LYM%	56.89	59.05	59.78	55.70	57.01	59.37
***p*-value**	0.574		0.316		0.554	
MID%	7.45	6.13	6.10	7.84	7.14	6.76
***p*-value**	0.197		0.069		0.734	
GRAN%	35.66	34.82	34.12	36.46	35.85	33.88
***p*-value**	0.794		0.506		0.535	
RBC 10^6/ul	5.07	5.25	5.24	5.12	5.12	5.35
***p*-value**	0.648		0.754		0.600	
HGB g/dl	8.51	9.58	9.28	8.78	8.83	9.60
***p*-value**	0.208		0.529		0.503	
HCT%	26.79	26.67	27.67	26.81	27.01	27.82
***p*-value**	0.956		0.686		0.716	
MCV fl	52.43	50.83	51.94	52.79	52.42	52.36
***p*-value**	0.247		0.463		0.963	
MCH pg	16.21	17.92	17.42	16.89	17.00	17.57
***p*-value**	**0.003***		0.377		0.460	
MCHC g/dl	31.56	36.38	33.88	32.30	32.73	33.97
***p*-value**	**0.003***		0.336		0.561	
RDW-SD fl	26.92	25.22	25.99	26.75	26.35	26.61
***p*-value**	0.189		0.534		0.883	
RDW-CV%	16.24	15.55	15.89	16.15	16.00	16.14
***p*-value**	0.196		0.604		0.848	

Discussion

In the present study the infection of babesia, theileria and their co-infection were detected. The overall Babesia infection rate was (35.4%) from the study population, (59.2%) for theileria and (23.1%) for their co-infection. The risk factors for these diseases studied here include: breeds, age and sex which may explain this differences. In current study the overall infection of babesiosis was relatively highly significant (0.007). Where the highest infection rate was in sheep (51.0%) then in cows (30.0%), and goats (20.5%). However, this study disagree with (Mohammed *et al*., 2018) who found Babesiosis infection rate higher in goats (60.77%) than sheep (46.70%) in Sennar state, Sudan. In this study the infection rate of Theileriosis was high (59.2%). Infection was found to be higher in sheep (64.7%) than goats (56.4%) and lowest in cows (55.0%). According to sex in cows, the female's rate was higher than the rate in males. This study agrees with (Abakar *et al.*, 2018) who found the prevalence rate of *T. annulata* antibodies in female (80%) was higher than in males (20%). In addition in this study, the infection rate according to age group was highest in cows between 1-3 years (70.6%), while he found the infection rate was highest in cows between 1-3 years (84.2%), and disagree with him

that the infection rate was followed by cows less than 1 year (50%) and the lowest was in cows more than 3 years (36.4%), while his study showed that the infection rate was followed by cows more than three years old (75.5%) and the lowest rate was in cows less than one years (60.8%) in Elhoush district in south Gezira State. In this study the prevalence of Theileriosis in sheep was higher in more than three years (66.7%) than the other categorical, this study agree with (Magzoub *et al.*, 2020) who found the highest prevalence rate was recorded in more than three years (21.6%) than the other. And disagree with him that the infection rate according to sex was higher in females (69.2%) than males (60%) while his study showed that the infection rate was higher in male (17.9%) than female (12.2%) in El Huda station in Al Gezira State and El Nuhud in West Kordofan State. However this study agree with (Ahmed *et al.*, 2018) who found infection rate was higher in female (83.3%) than male (52.4%) in Alhuda National Sheep Research Station, in Alhuda town. In this study the co-infection between Theileria and Babesia infection rate was higher in sheep (29.4%) than goats (15.4%), this study agree with (Mohammed *et al*., 2018) who found the co-infection between Theileria, Babesia and Anaplasma infection rate was higher in sheep (31.66%) than goats (18.62%) in Sennar state. In this study, Hematological analysis revealed haemato- Parameters values in Theileriosis in ruminants, which include decreases in the RBC count, WBC count, lymphocytes (LYM), packed cell volume (PCV), haemoglobin (Hgb) concentration, mean corpuscolar haemoglobin concentration (MCHC) with increased mean corpuscular volume (MCV), this result agree with (Agina *et al.*, 2020).who found the same result above in Holstein ruminants in Malaysia. In this study, Haematological analysis revealed haemato- Parameters values in Babesiosis in ruminants, expect of increasing in the RBC count, and haemoglobin (Hgb) concentration, this result disagree with (Abdalsalam, N.A. and Hazawy, S., 2017) who found Haematological analysis revealed haemato- Parameters values, expect of increasing in lymphocyte absolute count and mid-range absolute count (WBC), within the normal range in farm in ElWisata location in El-Gabal El-Akhdar area, El-Beida, Libya.

Conclusion:

Current study concluded that co-infection between Babesiosis and Theileriosis are detected in all types of ruminants tested. CBC test is useful in detecting infection by Babesiosis and Theileriosis, and co-infection especially in late stages of infection.

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