Sudan University of Science and Technology

College of Veterinary Medicine

Microscopic screening of blood parasite in East Nile Locality, Khartoum State, Sudan

مسح مجهري لطفيليات الدم في محلية شرق النيل، ولاية الخرطوم، السودان

A thesis Submitted in Partial Fulfillment for the Requirements for the Degree of B.Sc. in Veterinary Medicine

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إهداء

إلى من جرع الكأس فارغآ ليسقيني قطرة

حب

إلى من كلت أنامله ليقدم لنا لحظة سعادة

إلى من حصد الأشواك عن دربي ليمهد لي طريق العلم

إلى القلب الكبير (**والدي العزيز**)

إلى رمز الحب وبلسم الشفاء إلى الغالية التى لا نرى الأمل إلا من عينيها

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English Abstract

This study was carried out in East Nile locality, Khartoum State, Sudan as a screening study of blood parasites in cattle and sheep. For that, microscopic examination was applied. We have also investigated the effect of different blood parasites on some of the hematological parameters, namely, hemoglobin (Hb) and packed cell volume (PCV). Giemsa-stained blood smears reviled that, from 45 tested cattle in Selate and Kuku areas, 20 (44.5%) were positive for blood parasites, including *Babesia spp.* and/or other non-identified blood parasites. In 15 tested sheep in Diresab area, 7 (46.7%) were found to be positive for *Babesia spp.* and and/or other non-identified blood parasites. No significant differences between Hb concentration and PCV values were reported between the infected and non-infected animals. The current study demonstrated a high prevalence of blood parasites in the study areas. Further studies are anticipated to identify the parasite species.

Key Words: Blood parasites**,** East Nile locality*, Babesia spp*, *Theileria spp*, *Anaplasma spp*.

الملخص

في هذه الدراسة اجرى مسح مجهري لطفيليات الدم في الابقار والضان بمحلية شرق النيل بولاية الخرطوم وقد درس تأثير طفيليات الدم على بعض قياسات الدم مثل تركيز الهيموقلوين وحجم الخلايا المتراصة. اظهرت الدراسة أنه ومن بين 45 من الابقار التى خضعت للفحص المجهري أن 20(44.5)% كانت مصابه بطفيليات الدم بما في ذلك طفيل البابيزيا أو/و غيرها من طفيليات الدم التى لم يتم التعرف عليها مجهريا بينما اظهرت الدراسة أنه و من بين 15 من الضان التى تمت دراستها أن 7(46.7)% قد وجدت موجبة لطفيليات الدم بما في ذلك طفيل البابيزيا او/و طفيليات أخرى لم يتم تحديدها مجهريا. لم يتم تسجيل فرق معنوي في قياسات الدم بين الحيوانات المصابه وغير المصابه بطفيليات الدم. مزيدا من الدراسات متوقعه للتعرف على الطفيليات على مستوى النوع .

List of contents

List of tables

List of figures

List of abbreviations

T	Trypanosoma
A	Anaplasma
B	Babesia
T	Theileria
Hb	Haemoglobin
PCV	Packed Cell Volume
MOT	Malignant Ovine Theileriosis
R	Rhipicephalus
Ml	Mlliliters
EDTA	EthyleneDiamineTetraAcetic Acid
nmHb	Nanometre Haemoglobin
µL	Microlitres
gm/l	Gram per Liters
P	Prevelance
ELISA	Enzyme Linked ImmunosorbentAssay
PCR	Polymerase Chain Reaction

Introduction:

Cattle and sheep have a considerable economic importance as a source of animal proteins like milk and meat (FAO, 1992).

Many diseases may affect the productivity of those animals such as on these economic value as well as blood parasite as trypanosomosis, babesiosis, theileriosis and anaplasmosis. These diseases are caused by blood parasites.

African trypanosomosis, also known as nagana, is a devastating disease affecting both humans and animals in sub-Saharan Africa. The disease is caused by flagellated protozoa of the genus *Trypanosoma*, including *Trypanosoma brucei*, *Trypanosoma congolense*, and *Trypanosoma vivax* (Welburn et al. 2001).Babesiosis is a tick-born disease caused by protozoan parasites of the genus *Babesia*.It is affecting a wide range of domestic and wild mammalian hosts. Disease signs vary in severity from silent infection to acute circulatory shock with anemia, depending on susceptibility, immunity and age of the host, and on *Babesia species* and parasite load(Zintl, *et al* 2003, Homer, *et al* 2000 and Penzhorn, 2006). Worldwide, *Babesia spp* are primarily of veterinary importance (Zintl, *et al,* 2003 and L'Hostis, *et al,* 1995). The most important *Babesia spp*. that cause the disease in cattle are *Babesia bigemina*, *Babesia bovis* and *Babesia divergens* (Bouattour and Darghouth, 1996). In sheep the most important *Babesia spp* are *Babesia ovis* and *Babesia motasi* (Furmaga and Stanislaw, 1983).

Theilerosis is caused by an intracellular blood parasite it invades lymphocyte and macrophage as well. The most important *Theileria spp.* are *Theileria parva* that is known to cause east coast fever (Brayton, et al, 2007) and

Theileria anulata which causes tropical theileriosis in cattle. In sheep the disease is caused by *Theileria lestoquardi* (Gill et al., 1978).

Anaplasmosis is an infectious blood parasitic disease cause by *Anaplasma marginale* and *Anaplasma centrale* also new species of *Anaplasma*, *A. phagocytophilum* and *A. bovis* reported by Dumler *et al*., (2001)

All blood parasites have a considerable economic and veterinary medical impact worldwide that cause erythrocyte lysis lead to anemia, ictrus, haemoglobinuria, dyspnea and incoordination. Some of these diseases have an important zoonotic impact.

Objectives of the study:

The current study is aimed at screening the blood parasites in East Nile Locality using parasitological examination as well as investigating the relationship between the blood parasitic diseases and some hematological parameters, such as hemoglobin (Hb) and packed cell volume (PCV).

Chapter I

Literature review

Cattle and sheep are important multipurpose animals in Sudan.

Haemoprotozoan diseases especially trypanosomosis, babesiosis, theileriosis and anaplasmosis are considered as major impediments in the health and productive performance of cattle (Rajput *et al*., 2005).

Hemoprotozoan diseases cause devastating losses to the livestock industry throughout the world. However, it is known that most of blood protozoan parasites cause anemia by inducing erythrophagocytosis. Most of the hemoprotozoan parasites are tick borne parasites (Ananda *et al*., 2009). These diseases are wide spread in the Sudan (FAO, 1983) and cause substantial losses to the livestock industry throughout the world (Ananda *et al*., 2009; Kakarsulemankhel, 2011). These diseases have got their serious economic impact due to obvious reason of death, decreased productivity, lowered working efficiency (Uilenberg, 1995), increased cost for control measures (Makala *et al*., 2003) and limited introduction of genetically improved cattle in an area (Radostitis *et al*., 1994).

1.1. Trypanosomosis:

Bovine trypanosomosis (nagana) is a disease caused by extracellular protozon parasite *Trypanosma*, a *Tsetse*-transmitted parasites, and such as*T. congolense*, *T. vivax* and/or *T. brucei brucei* (Welburn et al. 2001).

Chronic wasting and anaemia are the most prominent features of animal trypanosomosis, but other pathologic effects are circulatory disturbances, leukopaenia,low serum complement levels, lymphoid tissue hyperplasia followed by hypoplasia and immunosuppression (Taylor andAuthie,2004). Trypanosomosis is consequently a severe constraint to animal agriculture in many parts of sub-Saharan Africa (McDermott and Coleman,2001;and Swallow,1998).

1.2. Babesiosis:

Babesia spp is a tick-borne protozoan parasite of cattle that causes serious economic losses in livestock industry (kuttler, 1988).

The most important causes of babesiosis in cattle are *B. bigemina*, *B. bovis*, *B. divergens* and *B. major*.

B. bigemina and *B. divergens* causes hemolytic babesiosis (synonyms: tick fever, red water and Texas fever). It is a disease characterized by hemolytic anemia, hyperpyrexia, hemoglobinuria, lethargy, anroxia and recumbency.

In severe cases cerebral babesiosis is manifested by several signs of central nervous system involvement and the outcome is almost invariably fatal (Bock *et al*., 2004).

Other causes of babesiosis in cattle is *B. bovis* and *B. major* that cause shock babesiosis. It is a disease characterize by high fever, ataxia and in coordination, anorexia, signs of general circulatory shock, production of dark red or brown colored urine, sometimes nervous signs associated with sequestration of infected erythrocytes in cerebral capillaries. Anemia and hemoglobinuria may appear later in the course of the disease.

Infected animals develop a life-long immunity against re-infection with the same species and some cross-protection is evident in *B. bigemina*-immune animals against subsequent *B. bovis* infections (World Health Organization for Animal Health, 2012).

1.3. Theileriosis:

The disease is caused by the apicomplexan protozoan parasite *Theileria spp*. that have a major effect on livestock health (Mehlhorn and Schein, 1984).

Bovine theileriosis is caused by *T. annulata* and *T. parva* which are round, ovoid rod-like or irregular shaped organism found in lymphocytes, histocytes and erythrocytes (Soulsby, 1982; Durrani *et al.,* 2008).

The disease caused by *T. parva*, known as east coast fever or Corridor disease, is one of the most serious cattle diseases in Eastern, Central, and Southern Africa (Conrad and Waldrup, 1993). The African buffalo is the natural reservoir host of *T. parva*, which is transmitted by the tick species, *Rhipicephalus appendiculatus*, *R. zambeziensis* and *R. duttoni* (Norval *et al*., 1991; Uilenberg, 1999).

T. lestoquardi is a highly pathogenic for sheep and the disease caused by the pathogen is known as malignant ovine theileriosis (MOT) and is transmitted by *Hyalomma* ticks (Levine, 1973).

The symptoms of theileriosis include high fever, listlessness, anorexia, emaciation, diarrhea or constipation, enlarged superficial lymph nodes, pale and icteric mucous membranes, anemia, difficult breathing and weight loss. An outbreak of theileriosis in sheep has been reported in Sudan (Tageldin *et al*., 1992).

Control of the disease can be achieved by chemotherapy, using theilericidal drugs such as buparvaquone (El Hussein *et al*., 1993) as well as tick control using acaricides (Jongejan and Uilenberg, 2004).

1.4. Anaplasmosis:

Outbreaks of bovine anaplasmosis are due to infection with *A. marginale. A. centrale* is capable of producing a moderate degree of anemia, but clinical outbreaks in the field are extremely rare. New species of *Anaplasma*, *A. phagocytophilum* and *A. bovis* (Dumler *et al*., 2001), with a primary reservoir in rodents, have been reported to infect cattle, but do not cause clinical disease (Dreher *et al.*, 2005; Hofmann-Lehmann *et al.*, 2004).

The most marked clinical signs of anaplasmosis are anemia and jaundice, the latter occurring late in the disease. Hemoglobinaemia and hemoglobinuria are not present, and this may assist in the differential diagnosis of anaplasmosis from babesiosis, which is often endemic in the same regions.

A. marginale causes bovine anaplasmosis which is characterized by the infiltration of the host's red blood cells. It can be transmitted to other hosts through mechanical transmission but the most important mode of transmission is via tick bites, the main tick vector being *R. decoloratus* (formerly *Boophilus decoloratus*) (Potgieter and vanRensburg, 1987; Potgieter and Stoltsz, 2004).*A. marginale* subsp. *centrale* causes a milder form of anaplasmosis, and is used in a live blood vaccine in many countries (Potgieter and van Rensburg, 1983).

Chapter II

Materials and Methods

2.1. Study area:

The samples were collected from several areas in East Nile locality, Khartoum State (Kuku, Selate and Diresab). These areas represent the intensive farms for milk and meat production in Khartoum State with a high probability of incidence of tick-born hemoprotozoan diseases.

2.2. Animals and Samples:

A total of 45 and 15 blood samples collected from cattle and sheep, respectively, were examined for blood protozoan parasites. Approximately 7 ml of blood samples were collected aseptically in sterilized syringes from the jugular vein and then aliquot into EDTA and plain containers. EDTA-treated blood was then used for hematological examinations such as Hb concentration and PCV. EDTA-free blood was used for serum preparation to be used in the future studies. Samples were transported to the laboratory in ice containers.

2.3. Preparation of Giemsa-stained blood smears:

One or two thin blood smears from each animal were prepared by ear vein puncturing, air-dried and fixed with methanol for 2-3 min. Smears were then stained with 10% Giemsa's stain for 20 min, then washed with clean water and examined under microscope (100 ×) with immersion oil for the

identification of blood parasites as described by Benjamin (1978) and Soulsby (1982).

2.4.PCV:

The microhematocrite tubes were filled till their two thirds to three-quarters with whole blood, one end was sealed by clay, trapping air between the blood and plug was avoided. Tubes were placed into a calibrated microhematocrit centrifuge with their sealed end out against the centrifuge rubber ring. The centrifuge was adjusted at 10,000 rounds per 5 minute, after centrifugation the blood was separated into three layers from the clay: red blood cells, white blood cells and platelets, and plasma. The hematocrite values were determined as a percent by using lined card (hematocrite ruler) , measuring the height of the total blood column and the height of the red cell layer as described by (Purves et al., 2004).

2.5. Hemoglobin (Hb):

Hb was evaluated using the spectrophotometer as described by (Tietz, 1976), using Hb measuring kit. Briefly, the working solution was prepared by adding 48 ml distilled water to 1ml potassium cyanide and 1ml potassium ferricyanide (Fig. 1).The three solutes were mixed together and from the mixture 2.5ml were placed in a clean cuvette. The cuvette was then placed in the spectrophotometer followed by the zero adjustment at the wavelength of 540 nm. Hb concentration for each sample was then measured by mixing 2.5ml of working solution with 10µlof whole blood in a clean cuvette. The cuvette was then placed in the spectrophotometer and the Hb value was

measured at the wavelength of 540 nm. Hb value was then normalized by being multiplied by the manufacturer value of 36.77 to give Hb in gm/l.

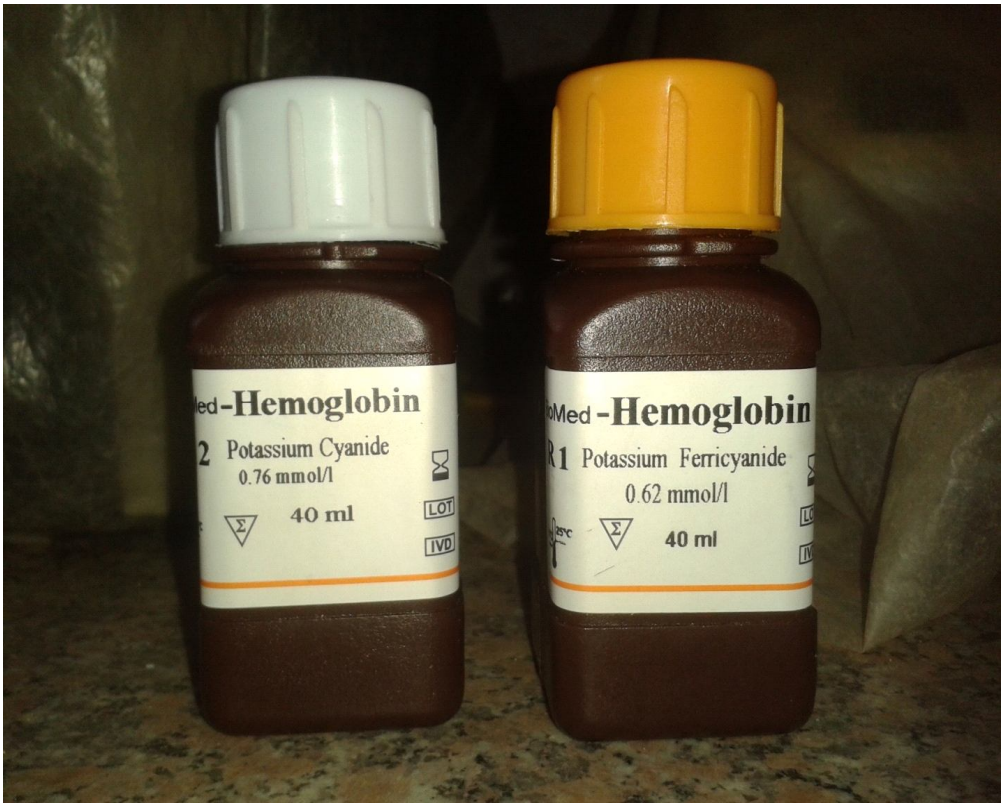

Fig. 1: The components of Hb measuring kit.

Chapter III

Results and Discussion

3.1. Blood parasite in cattle in Selate and Kuku areas:

3.1.1. Microscopic examination:

To investigate the presence of blood parasites, Giemsa-stained blood smears were prepared from 45 animals. Giemsa-stained blood smear reveal that 8 (17.8%) cattle were found to be positive for *Babesia Spp*. while 12 (26.7%) were found to be positive for others hemoparasitic parasites. The total number of the positive animals was 20 (44.5%) (Fig.2). The *Babesia spp*. were identified as a double form parasite. However, other single form parasites were also observe, these could be the single form of *Babesia spp.* (dividing stages) and/or other single form parasite such as *Theileria spp*. and *Anaplasma spp*. These data suggest that the tested animals could be infected with only *Babesia spp*, *Theileria spp*. or *Anaplasma spp*. Microscopically, we cannot rule out the possibility of mixed infection (Fig. 3). *Trypanosoma spp*. were not observed in any of the tested animals. This could be due to the low parasitemia that could not be detected by simple blood smears since it is known that *Trypanosoma* could be clearly observed in the smears prepared from the buffy coat. It is worth mentioning that the buffy coat technique was not applied in the current study.

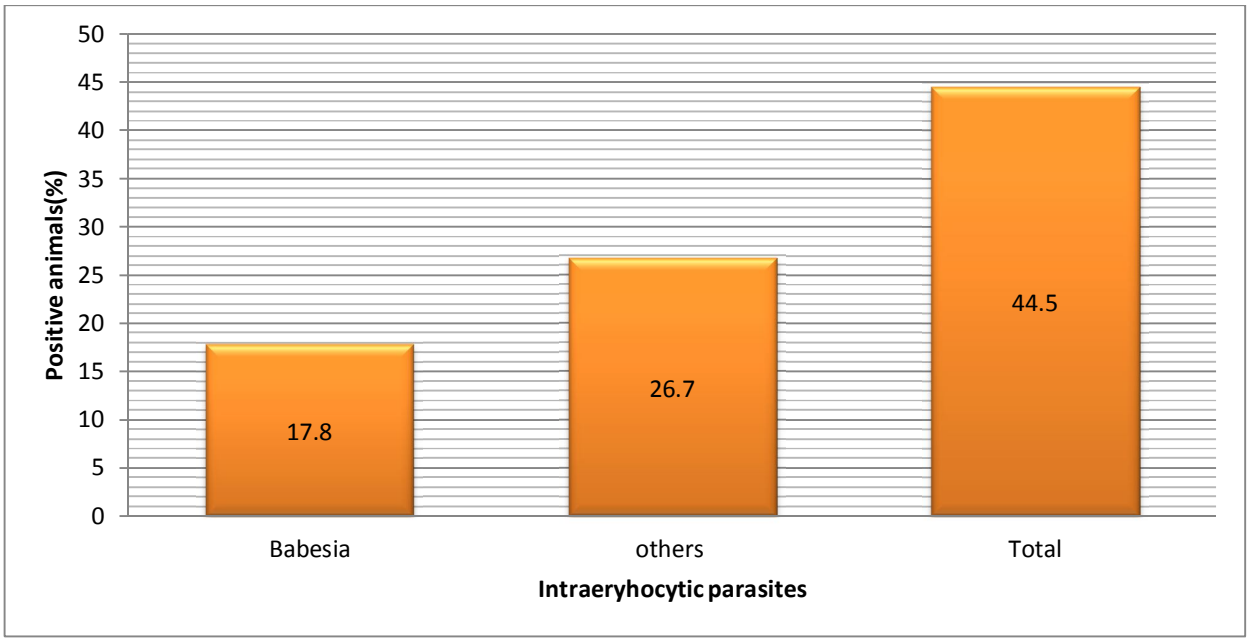

Fig. 2: Blood parasites in Selate and Kuku areas, East Nile locality, Khartoum State. From a total number of 45 blood samples, 8 cattle (17.8%) were identified as *Babesia spp*-positive and 12 (26.7%) were identified as positive for other intraerythrocytic parasites. The total number of infected animals with different parasites was 20 (44.5%).

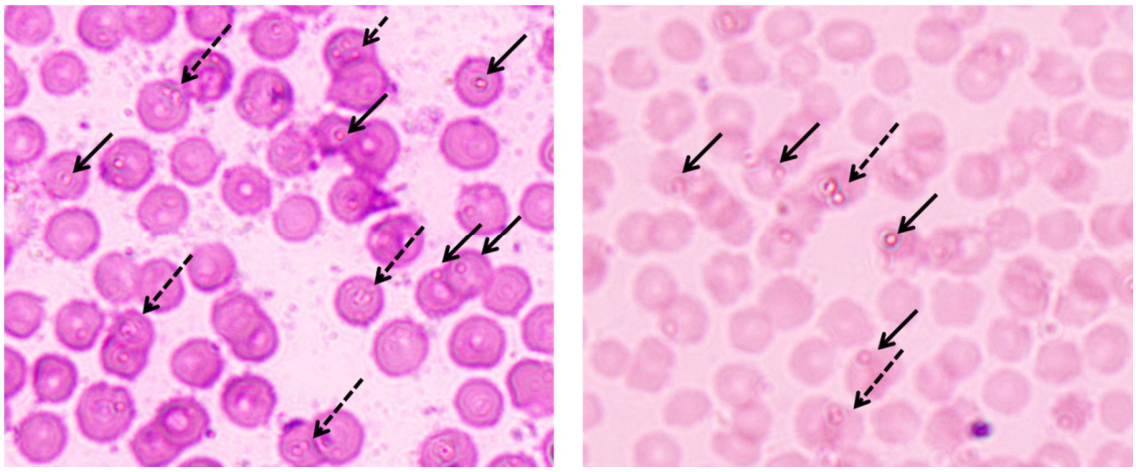

Fig. 3: Light microscopic observation of Giemsa-stained blood smears prepared from cattle in Selate and Kuku areas. Dashed arrows indicate the double form of *Babesia spp*. Solid arrows indicate the single form interaerythocytic parasites.

3.1.2. PCV:

The relationship between the diseases caused by blood parasites and the PCV values was investigated. It was found that PCV values were in the normal range in 19 samples collected from positive animals to blood parasites, while only one sample showed subnormal PCV value in the same group. On the other hand, all negative animals for blood parasites (25 animals) showed normal PCV values. There was no significant difference between PCV values in the infected and non-infected animals (Table.1). These data suggest that the infected animals with blood parasites could be at the early stages of the disease.

	Normal PCV	Subnormal PCV	Total
Positive animals for blood parasites	19	1	20
Negative animals for blood parasites	25	0	25
Total number of tested animals	44	1	45

Table. 1: PCV in infected and non-infected animals with blood parasites. The statistical significance of differences was assessed with *Chai*-square test. ($P>0.05$) between infected and non-infected animals.

3.1.3. Hb:

The relationship between the diseases caused by blood parasite and Hb was investigated. Hb concentration was measured using the spectrophotometer. Hb concentration was found to be in the normal range in 14 samples collected from positive animals to blood parasites, while 6 samples showed subnormal Hb value in the same group. On the other hand, 19 animals negative for blood parasites showed normal Hb concentration, while 6 animals negative for blood parasites showed subnormal Hb concentration. There was no significant difference between Hb concentration in the infected and non-infected animals (Table.2). These data suggest that the infected animals with blood parasites could be at the early stages of the disease, thus, they showed normal Hb concentrations. Moreover, the subnormal Hb concentration measured in the non-infected animals (6 animals) could be attributed to reasons other than blood parasites (Table.2).

	Normal HB	Subnormal Hb	Total
Positive animals for blood parasites	14	6	20
Negative animals for blood parasites	19	6	25
Total number of tested animals	33	12	45

Table. 2: Hb in infected and non-infected animals with blood parasites. The statistical significance of differences was assessed with *Chai*-square test, ($P>0.05$), between infected and non-infected animals.

3.2. Blood parasites in sheep (in Diresab area):

3.2.1. Microscopic examination:

To investigate the presence of blood parasites in one farm in Dresab area, Giemsa-stained blood smears were prepared from 15 animals. Giemsa-stained blood smears reveal that 2 (13.3%) sheep were found to be positive for *Babesia Spp*, 5 (33.3%) were found to be positive for others haemoparasitic parasites. The total number of the positive animals was 7 (46.7%) (Fig.4).The *Babesia spp.* were identified as a double form parasite. However, other single form parasites were also observe, these could be the single form of *Babesia spp* (dividing stages) and/or other single form parasite such as *Theileria spp* and *Anaplasma spp*. These data suggest that the tested animals could be infected with only *Babesia spp*, *Theileria spp*. or *Anaplasma spp*. Microscopically, we cannot rule out the possibility of mixed infection (Fig. 5).

The results of this study showed that infection with blood parasites were common in the selected farm (46.7% positive cases). That was most likely attributed to the miss management. It was observed that tick infestation was one of the great problems in the selected farm due to lack of application of proper management practices and misuse of acaricides as indicated by the owner. It was also observed that there was no routine screening for blood parasites. A previous study carried out by Abdelbasit, (2006), reported a lower prevalence of ovine babesiosis (15.9%) as compared to our study. They have examined a group of animals brought to the slaughter house from different parts of Sudan. In contrast, the high prevalence of babesiosis in our study, as compared to the previous one could be attributed to the fact that we

have examined animals confined in one farm with clinical symptoms. Moreover, variation in geo-climatic condition, breed, and exposure of vectors and age of the animals might contribute to variable prevalence of hemoprotozoan diseases in both studies (Muhanguzi *etal.,* 2010).

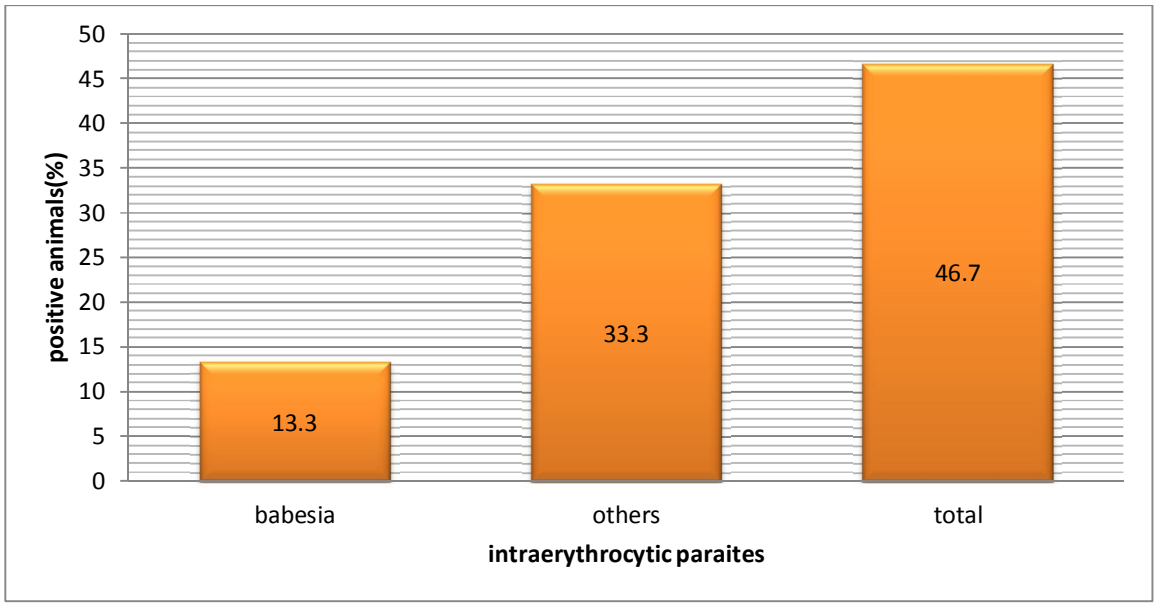

Fig. 4: Blood parasites in Diresab area. From a total number of 15 blood smears prepared from sheep,2(13.3%) were identified as *Babesia spp* and 5 (26.7%) were identified as other intraerythrocytic parasites. The total number of infected animals with different parasites was 7 (46.7%).

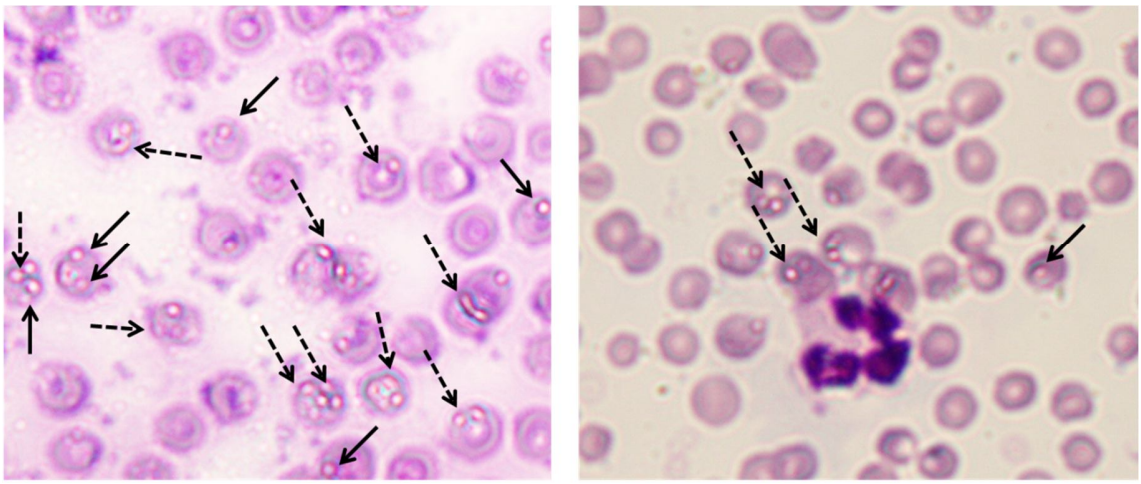

Fig. 5: Light microscopic observation of Giemsa-stained blood smears prepared from sheep in Diresab area. Dashed arrows indicate the double form of *Babesia spp*. Solid arrows indicate the single form interaerythocytic parasites.

3.2.2. PCV:

The relationship between the diseases caused by blood parasites and the PCV values was investigated. It was found that PCV values were in the normal range in 4 samples collected from positive animals to blood parasites, while 3 samples showed subnormal PCV value in the same group. On the other hand, 6 negative animals for blood parasites showed normal PCV values, while 2 negative for blood parasites showed subnormal PCV values. There was no significant difference between PCV values in the infected and non-infected animals. (Table.3). these data suggest that the infected animals with blood parasites could be at the early stages of the disease.

	Normal PCV	Subnormal PCV	Total
Positive animals for blood parasites	4	3	7
Negative animals for blood parasites	6	2	8
Total number of tested animals	10	5	15

Table. 3. PCV in infected and non-infected sheep with blood parasites in Diresab area. The statistical significance of differences was assessed with *Chai*-square test, (P>0.05), between infected and non-infected animals.

3.2.3.Hb:

The relationship between the diseases caused by blood parasite and Hb was investigated. Hb concentration was measured using the spectrophotometer. Hb concentration was found to be in the normal range in one sample collected from positive animals to blood parasites, while 6 samples showed subnormal Hb value in the same group. On the other hand, no one of the negative animals for blood parasites showed normal Hb concentration. There was no significant difference between Hb concentration in the infected and non-infected animals (Table.4). In general, in both infected and non-infected animals we observed that Hb concentration was notably subnormal These data suggest in the tested farm, in general there was a common reason, not only the blood parasites caused the low Hb concentration in the infected and non infected animals. This most probably could be nutritional reasons.

	Normal Hb	Subnormal Hb	Total
Positive animals for blood parasites	1	6	7
Negative animals for blood parasites	0	8	8
Total number of tested animals	1	14	15

Table. 4: Hb in infected and non-infected sheep with blood parasites in Diresab area. The statistical significance of differences was assessed with *Chai*-square test, (P>0.05), between infected and non-infected animals.

Conclusion:

In the current study we have screened microscopically the prevalence of blood parasites in cattle and sheep in East Nile Locality, Khartoum State, Sudan. We have reported that 44.5% of the tested cattle were infected with different blood parasites, while 46.7% of tested sheep were found to be positive for different blood parasites. These include *Babesia spp* and other not-characterized intraerythrocytic parasites. The latter might include *Thieleria spp* and *Anaplasma spp*. These high percentages of infection indicate that these diseases are endemic in the study areas. Moreover, the data we have obtained in this study do not reflect the real prevalence of these diseases since we have used only microscopic examination. Other more sensitive techniques like Enzyme Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) might detect a higher prevalence.

As it is known that hemoparasitc diseases cause disturbance in the hematological parameters of the infected animals (Ananda *et al*., 2009 and Muraleedharan, *et al*., 2005) due to the damage to the erythrocytes (Mehta *et al*., 1988), we have investigated some hematological parameters including Hb concentration and PCV in the microscopically positive animals as compared to the negative ones. We found that there was no significant difference between the parasite-positive and parasite-negative animals in terms of Hb and PCV. This could be attributed to the fact that the infected animals might be at the early stages of the disease.

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