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Prevalence and Risk Factors of Equine Piroplasmosis in Khartoum State, Sudan

الإنتشار وعوامل الخطر لداء بايروبلازما الخيل بولاية الخرطوم، السودان

A thesis submitted for partial fulfillment of the requirements of the degree of Master in Preventive Veterinary Medicine (MPVM)

By

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Dedication

To my parents

To my brothers and sisters

Acknowledgements

Firstly, praise and thanks to Almighty Allah for giving me strength to accomplish this work. I would like to express my sincere gratitude and appreciation to my supervisor Dr. Naglaa Abd El Hakeem Abass for her continuous motivation and advice.

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Abstract

A cross-sectional study was conducted in the period from October 2016 to July 2017 to determine the prevalence of equine piroplasmosis in horse population in Khartoum State, Sudan, using Giemsa stained thin smears. Moreover, epidemiological factors that associated with the occurrence of the disease including: location, sex, age, breed, body condition score, horse use, housing system, previous infection with piroplasmosis, previous treatment, tick infestation, use of acaricides, contact with other animal species, biological control of ticks and season of the year were also investigated.

Giemsa staining method revealed that the overall prevalence of equine piroplasmosis in horses was 27.4% (75/274) with geographical variation: 35.5% in Khartoum, 9.5% in Um durman and 25% in Khartoum North. Univariate analysis using Chi-square test at $P \leq 0.05$ significance showed positive association between prevalence of the disease and geographic location ($x^2 = 15.345$, $P = 0.000$), sex ($x^2 = 9.428$, $P = 0.002$), age ($x^2 = 7.260$, $P = 0.027$), horse use ($x^2 = 16.949$, $P = 0.000$), previous treatment ($x^2 = 11.115$, $P = 0.001$), tick infestation ($x^2 = 4.363$, $P = 0.037$), use of acaricides ($x^2 = 5.144$, $P = 0.023$), biological control of ticks ($x^2 = 4.210$, $P = 0.040$) and season of the year ($x^2 = 8.268$, $P = 0.016$). Breed ($x^2 = 1.076$, $P = 0.300$), body condition score ($x^2 = 0.079$, $P = 0.778$), housing system ($x^2 = 1.049$, $P = 0.306$) and contact with other animal species (x^2 0.069, $P = 0.793$) were found not associated with the prevalence of the disease.

The present study confirmed that equine piroplasmosis was endemic in horses in Khartoum State, Sudan and the control of the disease should be focused on control of ticks and treatment of carrier animals.

ملخص

أجريت دراسة عرضية من أكتوبر 2016 إلى يوليو 2017 لتحديد نسبة إنتشار داء البايروبلازما في الخيل بولاية الخرطوم – السودان بإستخدام مسحات رقيقة مصبوغة بجيمسا. علاوة على ذلك، تم التقصي عن العوامل الوبائية المرتبطة بحدوث المرض: الموقع، الجنس، العمر، السلالة، مستوى حالة الجسم، إستخدام الحصان، نظام الإسكان، الإصابة السابقة بالبايروبلازما، المعالجة السابقة، الإصابة بالقراد، إستخدام مبيدات القراد، الإحتكاك مع نوع حيوان اخر، المكافحة البيولوجية للقراد وموسم الإصابة.

أظهرت صبغة جيمسا أن الإنتشار الكلي لبايروبلازما الخيل يعادل 27.4% (274\75) مع وجود تباين جغرافي: الخرطوم 35.5%، أمدرمان 9.5% والخرطوم بحري 25%. التحليل أحادي المتغير بإستخدام إختبار مربع كاي عند مستوي $P \leq 0.05$ أظهر وجود إرتباط بين تكرار المرض والموقع الجغرافي ($x^2 = 15.345, P = 0.000$)، الجنس ($x^2 = 9.428, P = 0.002$)، العمر ($x^2 = 7.260, P = 0.027$)، إستخدام الحصان ($x^2 = 16.949, P = 0.000$)، المعالجة السابقة ($x^2 = 11.115, P = 0.001$)، الإصابة بالقراد ($x^2 = 4.363, P = 0.037$)، إستخدام مبيدات القراد ($x^2 = 5.144, P = 0.023$)، المكافحة البيولوجية ($x^2 = 4.210, P = 0.040$)، وموسم الإصابة ($x^2 = 8.268, P = 0.016$). وجد أن السلالة ($x^2 = 1.076, P = 0.300$)، مستوى حالة الجسم ($x^2 = 0.079, P = 0.778$ =)، نظام الإسكان ($x^2 = 1.049, P = 0.306$) والإحتكاك مع نوع حيوان اخر ($x^2 = 0.069, P = 0.793$) غير مرتبطة بإنتشار المرض.

أكدت هذه الدراسة أن داء بايروبلازما الخيل متوطن في الخيل بولاية الخرطوم، السودان ويجب أن يرتكز برنامج مكافحة المرض على مكافحة القراد ومعالجة الحيوانات الحاملة للمرض.

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Introduction

Equine piroplasmosis is a tick-borne protozoan disease of horses, donkeys, mules and zebra. (OIE, 2014). The causative agents are two distinct apicomplexan protozoan parasites *Babesia caballi (B. caballi)* and *Theileria equi (T. equi)*. They are transmitted by ticks of the genera *Dermacentor, Hyalomma* and *Rhipicephalus* (Kamyingkird *et al*., 2014).

Equine piroplasmosis spreads in southern Europe, Asia, countries of the Commonwealth of Independent States, Africa, Cuba, South and Central America, and certain parts of the Southern United States of America. *T. equi* has also been reported in Australia, and is now believed to have a wider general distribution than *B. caballi* (OIE, 2014).

Clinically infected horses show signs of fever, increased respiratory and heart rates accompanied with loss of appetite, sweating, congested membranes with petechial hemorrhages. Diseased horses also suffer from panting with labored respiration, depression, passing of brownish coffee-like urine; moreover, colicky signs have accompanied with diarrhea and/or constipation (Oladipo *et al*., 2015).

Equine piroplasmosis can be diagnosed by clinical diagnosis, serological tests and molecular diagnosis. Clinical diagnosis is based on observation of clinical signs and the microscopic examination, but clinical signs may be confused due to the variety of other conditions. Microscopic examination is known to be of low sensitivity. The serological tests include complement fixation test (CF), indirect fluorescent antibody test (IFA) and enzyme-linked immunosorbent assay (ELISA). Serological tests are more reliable and capable of detecting antibodies against the parasites (Kamyingkird *et al*., 2014).

Despite, serological tests provide evidence of antibodies against parasites but these tests do not distinguish between the past and current infections. Therefore, combination of molecular and serological detection for the infection provides powerful tools for accurate diagnosis as well as for epidemiological investigation (Kamyingkird *et al*., 2014).

Currently, hundreds of horses are habited in Khartoum State. However, few epidemiological studies have been conducted on equine piroplasmosis in the region. The prevalence of equine piroplasmosis in donkeys in Khartoum State was 8.3% by

Giemsa's staining (Nada, 2010). Salim *et al*., (2013) reported that the overall prevalence of the disease among horses and donkeys in the Sudan was 35.95% using molecular techniques. Therefore, there is a strong need to carry out further epidemiological research on equine piroplasmosis in Khartoum State.

Objectives:

1. To update the prevalence of equine piroplasmosis in horse population using Giemsa's staining in Khartoum State, Sudan.

2. To identify the risk factors of the disease among horses in the study area.

CHAPTER ONE

LITERATURE REVIEW

1.1 Definition

Equine piroplasmosis (EP) is a tick-borne protozoal disease of horses, mules, donkeys and zebra. The causative agents are blood parasites named *Theileria equi (T. equi)* and *Babesia caballi (B. caballi)*. Infected animals may remain carries of these parasites for long times and act as sources of infection for ticks, which act as vectors (OIE, 2014).

1.2 Taxonomy of *Babesia caballi* and *Theileria equi*

Babesia caballi and *Theileria equi* belong to genera Babesia and Theileria, and the families Babesiidae and Theileridae, respectively and order Piroplasmida (Table 1.1) (Soulsby, 1982).

1.3 Geographic distribution

The causative agents of the disease are endemic in many tropical and subtropical regions of the world, as well as temperate climate zones including southern Europe, Asia, Africa, South and Central America, the Middle East and the Caribbean (Liv, 2009; Salim *et al*., 2013; Al-Obaidi *et al*., 2016).

In Central-Southern Italy, the overall seroprevalence of *T. equi* was 39.8% in horses, while molecular prevalence was 70.3%. For *B. caballi*, the overall seroprevalence was 8.9% and its molecular prevalence was 10.3% (Leticia *et al*., 2016).

Liv (2009) recorded that the seroprevalence of *T. equi* was significantly higher (4.4%) than that of *B. caballi* (1.5%) in the Swiss horse population.

The disease was also detected among horses and mules from North Thailand by Enzyme-Linked Immunosorbent Assay (ELISA) with an overall prevalence of 2.5% and 5.42% for *B. caballi* and *T. equi*, respectively (Kamyingkird *et al*., 2014). Likewise, the disease was also reported in horses in Brazil (Kerber *et al*., 2009), in equines in Pakistan (Javed *et al*., 2014) and in Persian Arab horses from Iran (Bahrami *et al*., 2014).

Table 1.1: Taxonomy of *Babesia caballi* and *Theileria equi* (Adapted from Soulsby, 1982).

Subclass	Piroplasmia
Order	Piroplasmida
Family	Babesiidae, Theileriidae
Genus	Babesia, Theileria

In Africa, Oduori *et al*., (2015) found that the overall seroprevalence of *T. equi* in donkeys from Nuu Division, Kenya was 81.2% using competitive ELISA (cELISA), while antibodies against *B. caballi* were not detected. Low proportion of babesiosis (1.75%) was reported in working donkeys in Adigudem and Kwiha districts of Tigray region, Northern Ethiopia (Mekibib *et al*., 2009).

Using PCR amplification of the 18S rRNA gene, Salim *et al*., (2013) reported that the overall prevalence of equine piroplasmosis in the Sudan was 35.95%. 206 samples from horses and 102 from donkeys were collected from Kassala, Gadaref and North Kurdofan States. *T. equi* infections were found in all investigated areas and no *B. caballi* infection was detected. The highest prevalence was found in Gadaref State (72.9%), followed by Buram (50%) and Kassala (26.9%). The lowest prevalence was recorded in Nyala and Shearia, 5.9% and 5.6%, respectively.

1.4 Transmission

Equine piroplasmosis is a tick-borne infection of equids and its occurrence needs competent vector. The principle hosts of EP are horses, mules, donkeys and zebra. Affected equids with causative parasites of the disease may remain carriers for long periods and act as sources of disease for other vectors (OIE, 2009).

Ixodid ticks of the genera *Dermacentor, Rhipicephalus* and *Hyalomma* are vectors of *B. caballi* and *T. equi. B. caballi* invade various organs of ticks and transmit transovarially from egg to larva, while *T. equi* develops only in salivary glands of vectors and do not found in other tick organ. Transmission is also possible through mechanical vectors contaminated by infected blood (OIE, 2009).

1.5 Life cycle

In erythrocytes, *B. caballi* sporozoites transform into trophozoites which grow and divide into two; oval, round or pear-shaped merozoites, which in turn, are capable of infecting new erythrocytes. *T. equi* sporozoites inoculated into horse via a tick-bite invade the lymphocytes and undergo development and form schizonts; merozoites released from these schizonts invade erythrocytes and transform into trophozoites which grow and divide into Maltese-cross merozoites (OIE, 2009).

When a tick feeds on an infected equid, infected erythrocytes containing merozoites or gametocytes are taken up by the tick (Michael, 2016). Most of the parasites

deteriorate in the gut lumen of the tick, but some develop further to undergo sexual reproduction. The resulting zygotes of *B. caballi* infect cells of various organs of the vector in which they divide again, this process persists in the next tick stage by transstadial transmission. In the next tick stage, the sporozoites are released into the saliva to infect the horse. Transovarial transmission may take place in the adult female tick (Rùegg, 2008).

Without multiplication in the endothelial cells of the tick gut, the zygotes of *T. equi* directly invade the hemolymph where they move to the salivary glands cells, and when the tick feeds on a new horse, matured sporozoites are inoculated in the dermis along the vectors saliva during the final hours of feeding. The infection rate of equine piroplasmosis is associated with the length of the time the vector is attached to the horse and which amounts 100% if the vector completed feeding cycle (Michael, 2016).

1.6 Pathogenesis and clinical signs

During the infection with *T. equi* and *B. caballi*, parasitemia rarely exceeds 5% and both the course of infection and the clinical signs depend on the status of the immune system of the equid and the virulence of the etiological agent (Rùegg, 2008).
The incubation period of the equine piroplasmosis is 1-3 weeks. The primary effect of disease is hemolysis and the infected equid suffer from paleness of mucous membranes, jaundice, enlargement of spleen, hypotension and blooded urine. Complications including multiple organ failure may develop like acute renal failure, renal damage, hemolytic anemia, pulmonary edema and cerebral dysfunction (Michael, 2016).

1.7 Diagnosis

Equine piroplasmosis can be diagnosed by a combination of clinical signs, microscopic examination, serological testing, polymerase chain reaction (PCR) and inoculation of blood into a susceptible host (Janina, 2008).

1.7.1 Clinical diagnosis

The clinical diagnosis of the equine piroplasmosis can easily be confused with other conditions because the signs of equine piroplasmosis are often nonspecific. The disease should be differentiated from surra, equine infectious anemia, dourine, African horse sickness, purpura haemorrhagica and plant and chemical toxicities (OIE, 2014).

1.7.2 Microscopic examination

Infection can be confirmed by demonstrating the parasites in stained blood or organ smears during the acute phase of the disease using Romanovsky-type staining methods, such as Giemsa.

It is extremely difficult to detect piroplasms especially of *B. caballi* infections in low parasitaemic carrier hosts. Paired merozoites joined at the posterior ends are a characteristic feature of *B. caballi*, while the arrangement of the pear-shaped merozoites forming a tetrad known as a (Maltese cross) is the characteristic one of *T. equi* (OIE, 2014).

1.7.3 Serological tests

Indirect fluorescent antibody test (IFAT) and Enzyme-linked immunosorbent assay (ELISA) are recommended for international trade and the successful differential diagnosis of piroplasms *T. equi* and *B. caballi* infections. Indirect ELISA using recombinant *T. equi* and *B. caballi* proteins showed high sensitivity and specificity in detecting antibodies in affected horses.

In some countries, Complement fixation test (CFT) has been used by to qualify horses for importation, but it is less sensitive than IFAT and ELISA (OIE, 2009).

1.7.4 Molecular techniques

Species-specific polymerase chain reaction (PCR) tests, targeting the 18s rRNA gene as well as BC48 (*B. caballi*) and EMA-1 (*T. equi*) genes, are available for molecular detection of *B. caballi* and *T. equi*. These techniques are highly specific and sensitive (OIE, 2014).

1.8 Treatment

B. caballi infection in horses is treated with administration of imidocarb dipropionate injection at 2.2 mg/kg intramuscular injection for 2 doses with an interval of 24-48 hours. Sterilization of *Babesia caballi* infection would be accomplished by 4 intramuscular injections of 4 mg/kg imidocarb dipropionate at 72 hour intervals. Treatment and clearance of *T. equi* infection of horses are achieved by 4 intramuscular injections of 4 mg/kg imidocarb dipropionate at 72 hour intervals (Michael, 2016).

Diminazene aceturate deep intramuscular injection can be used at 11 mg/kg 2 doses with an interval of 24 hours to eliminate *B. caballi* infection (Michael, 2016).

1.9 Prevention

There is no vaccine available for either *B. caballi* or *T. equi.* Prevention of infection can be achieved by tick control and treatment of carrier animals.

To prevent the introduction into disease-free countries, horses should be tested by IFA or ELISA before movement. Positive horses to equine piroplasmosis should be quarantined from both free horses and vectors. Because of the possible iatrogenic transmission of equine piroplasmosis with contaminated blood, prevention measures should be taken (OIE, 2014).

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study area

This study was conducted in the period from October 2016 to July 2017 in Khartoum State, a north eastern part of the central Sudan. The area is located between latitude 15° 32.799´N and longitude 32° 32.0166´ E in an area about 22.122 km^2 and is geographically divided into seven localities (Khartoum, Jabal Aulya, Um durman, Karari, Um badah, Khartoum North and Sharq El Nile). The state has a semi-arid climate with mean annual rainfall of 156.8 mm. The mean annual minimum and maximum temperatures are 22.7° C and 37.1° C, respectively (Figure 2.1).

2.2 Study design and sample size

Cross-sectional design was selected to fulfill the study objectives. The sample size was calculated according to the formula described by Thrusfield (2007) using a 35.95% expected prevalence (Salim *et al*., 2013) and 95% level of confidence interval (CI) with 5% desired absolute precision. The estimated sample size was 354 but a number of horses' owners refused participation in this study so the total collected blood samples were 274.

2.3 Sampling method and study animals

In Khartoum State, part of horse population is kept in stables in various districts. Numbers of these horses are patrolling horses (riding) and the others are horses that used in sport (racing and jumping horses kept in private stables). Multistage random sampling strategy was followed to collect blood samples from the study animals and all localities in the State were surveyed during the study period. Within the 7 localities of the State, horses' stables were further conveniently selected. A total of 25 stables that have at least one horse were visited and horses were sampled during the visit.

In the current survey, examined horses were from all ages, both sexes, including indigenous and crossbred and their body condition state was divided into thin and good scores according to Henneke system (Henneke *et al.*, 1983).

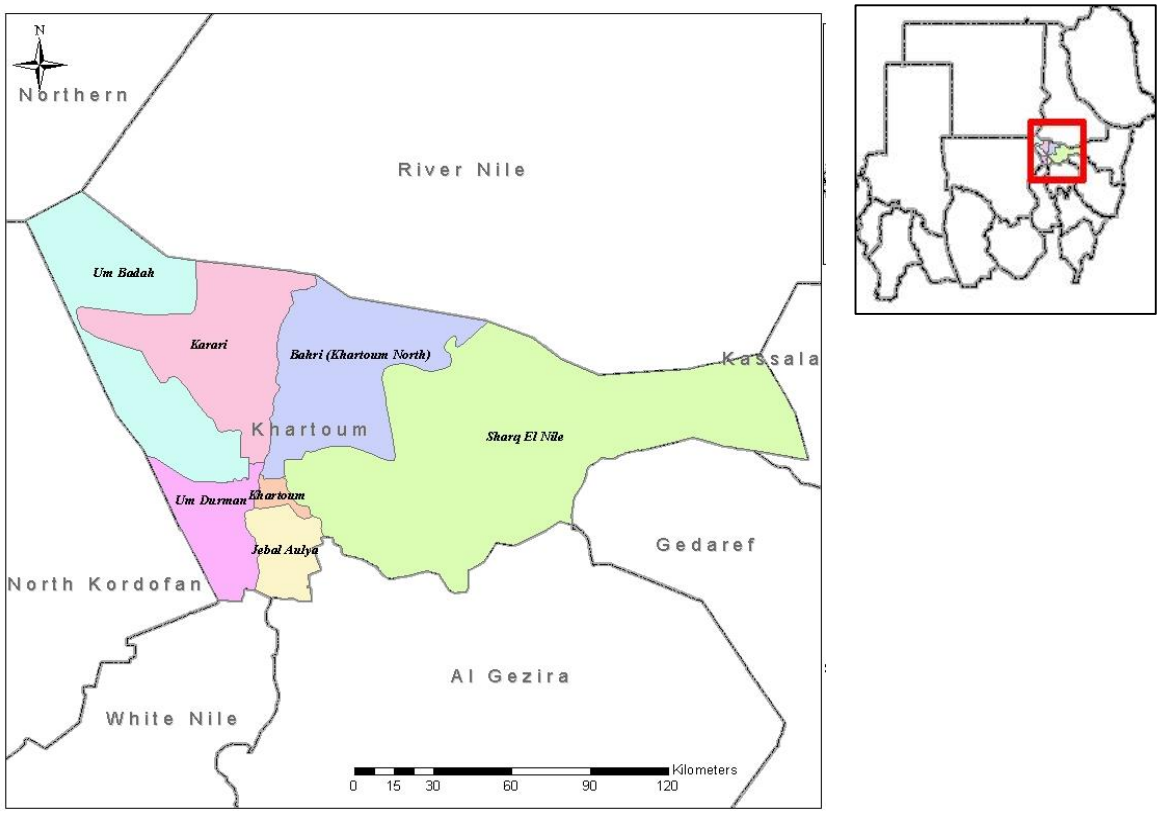

Figure 2.1: Map of 7 sampled localities in Khartoum State

2.4 Sample collection

274 blood samples were collected from apparently healthy horses located in studied areas. After disinfection, approximately 5 ml blood was drawn from jugular vein with the help of a sterilized syringe. The collected blood samples were transferred immediately into tubes containing ethylene diamine tetra acetic acid (EDTA). Blood samples were kept in cooled condition and immediately transported to the laboratory. Blood smears were prepared and examined by light microscopy in the same day of blood collection.

2.5 Blood smear

From blood samples, blood smears were prepared according to OIE (2014) as follows; a small drop of blood on to a clean glass slide was placed then air-dried and heat-fixed at 80°C for 5 minutes, and stained with 5% Giemsa (ATOM SCIENTIFIC, Manchester, UK) for 20–30 minutes. Stained blood smears were screened for the presence of intracellular parasites under 100x oil immersion lens of light microscope.

2.6 Data collection

Data considering disease determinants: location, sex, age, breed, body condition score, horse use, housing system, previous infection (clinical signs) with piroplasmosis, previous treatment, tick infestation, use of acaricides, contact with other host species, biological control and season were obtained using a predesigned questionnaire for each examined horse (Appendix 1).

2.7 Statistical analysis

All data were coded and entered into an Excel spreadsheet (Microsoft Excel, 2016). Data were analyzed with the statistical package for the social sciences (SPSS) version 16.0 software (SPSS Inc., Chicago, IL, USA). Prevalence and frequencies were calculated as percentages. Chi-squared test was used to identify associated risk factors with piroplasmosis and the level of significance was set at p-value ≤ 0.05.

CHAPTER THREE

RESULTS

3.1 The Prevalence of equine piroplasmosis in horses in Khartoum State

In this study, a total of 274 blood samples were obtained from horses in 25 stables from 7 different localities of Khartoum State and tested with Giemsa's stain (Figure 3.1). Accordingly, the overall prevalence of equine piroplasmosis in horses in the State was 27.4% (75/274).

Geographically, the distribution of 274 examined horses was 56.6% (155), 23% (63) and 20.4% (56) in Khartoum (including Khartoum and Jabal Aulya localities), Um durman (including Um durman, Karari and Um badah localities) and Khartoum North (including Bahri and Sharq El Nile localities) areas, respectively. The highest prevalence of equine piroplasmosis was found in Khartoum area 35.5% (55/155) followed by Khartoum North 25% (14/56) and the lowest one was noted in Um durman 9.5% (6/63) (Table 3.1).

3.2 Risk factors analysis

Based on the results, 22.4% (45/201) stallions and 41.1% (30/73) mares were found to be positive for equine piroplasmosis. Chi-squared test showed association in the prevalence of equine piroplasmosis between stallions and mares ($x^2 = 9.428$; $P = 0.002$) (Table 3.2).

Out of 274 examined horses, 8.4% (23) were 1-3 years old, 19% (52) (>3)-5 years old and 72.6% (199) >5 years old. Prevalence of equine piroplasmosis was 21.7% (5/23), 42.3% (22/52) and 24.1% (48/199) in 1-3, (>3)-5 and >5 age groups, respectively. Accordingly, association in the prevalence of equine piroplasmosis among different age groups was detected ($x^2 = 7.260$, $P = 0.027$) (Table 3.2).

39 out of 274 surveyed horses (14.2%) were crossbred while 85.8% (235/274) were local breeds. Prevalence of equine piroplasmosis was 28.5% (67/235) in local breed and 20.5% (8/39) in crossbred horses. Therefore, in terms of breed, there was no association between the prevalence of equine piroplasmosis and breed of tested horses ($x^2 = 1.076$; $P = 0.300$) (Table 3.2).

Studied population was composed of 4.7% (13/274) horses that scored thin body condition but the rest 95.3% (261/274) had a good body condition The prevalence of equine piroplasmosis was higher in thin horses 30.8% (4/13) than good ones 27.2% (71/261). Chi-squared test revealed absence of association between the prevalence of equine piroplasmosis and variant body condition scores ($x^2 = 0.079$; $P = 0.778$) (Table 3.2).

Of 274 sampled horses, 61.7% (169) were used in riding, 34.3% (94) were used in sport and 4% (11) were used for other activities (dragging and breeding). Prevalence of equine piroplasmosis was 18.9% (32/196), 42.6% (40/94) and 27.3% (3/11) in riding, sport and both dragging and breeding horses, respectively. Therefore, horse use was identified as a risk factor for equine piroplasmosis in the present study ($x^2 = 16.949$, $P = 0.000$) (Table 3.2).

36.9% (101) of 274 tested horses were kept in groups in open yards, while 63.1% (173) were kept individually in separate stables. Equine piroplasmosis was more prevalent in horses kept in separate stables 29.5% (51/173) than horses kept in open yards 23.8% (24/101). There is no association between prevalence of equine piroplasmosis and two different housing systems ($x^2 = 1.049$; $P = 0.306$) (Table 3.2).

Prevalence of equine piroplasmosis was 36.4% (8/22) in horses that have suffered from previous infection (clinical signs) with equine piroplasmosis and 26.6% (67/252) in horses that have not suffered from previous infection (clinical signs) with equine piroplasmosis. Chi-squared test revealed no association between the two categories ($x^2 = 0.973$; $P = 0.324$) (Table 3.2).

Prevalence of equine piroplasmosis was 23.6% (55/233) in horses that were not subjected to previous treatment of equine piroplasmosis, while the prevalence of equine piroplasmosis was higher 48.8% (20/41) in horses that were subjected to Imidocarb treatment. Chi-squared test showed association between the prevalence of equine piroplasmosis and the two groups ($x^2 = 11.115$; $P = 0.001$) (Table 3.2).

Concerning tick infestation, prevalence of equine piroplasmosis was higher in horses that were not infested by ticks 32.5% (49/151) than other horses that were infested by ticks 21.1% (26/123) (Table 3.2).

Cypermethrin was used for the chemical control of ticks in 95.3% (261/274) of investigated horses, while 4.7% (13/274) were not exposed to Cypermethrin or any other type of chemical control. Prevalence of equine piroplasmosis was 28.7% (75/261) and 0% in cases of disease that exposed to acaricides and piroplasmosis cases that not exposed to acaricides, respectively. There was an association between equine piroplasmosis and use of acaricides ($x^2 = 5.144$; $P = 0.023$) (Table 3.2).

The prevalence of equine piroplasmosis was 25.6% (10/39) in horses that kept in close contact with donkeys and 27.7% (65/235) in horses reared without contact with other animal species. Chi-squared test indicated no association between the prevalence of equine piroplasmosis and contact with other animal species ($x^2 = 0.096$; $P = 0.793$) (Table 3.2).

Only 17 (6.2%) of examined horses were reared with local breeds of chicken to control ticks biologically and the majority of horses 257 (93.8%) were not exposed to this type of control. Accordingly, the prevalence of equine piroplasmosis was 28.8% (74/257) in horses raised without biological control and 5.9% (1/17) in horses that were kept with chicken. Results summarized in Table 3.2 denoted an association between the prevalence of equine piroplasmosis and biological control of ticks ($x^2 = 4.210$; $P = 0.040$) (Table 3.2).

Horses were sampled during all seasons of the year; 66 (24.1%), 97 (35.4%) and 111(40.5%) horses were examined during fall, summer and winter, respectively. The highest prevalence of equine piroplasmosis was recorded in Fall 40.9% (27/66), followed by Summer 24.7% (24/97) and the lowest prevalence of equine piroplasmosis was recorded in Winter 21.6% (24/111) with association between the prevalence of equine piroplasmosis and season of the year ($x^2 = 8.268$, $P = 0.016$) (Table 3.2).

Table 3.1: Prevalence of equine piroplasmosis in horses in Khartoum State

District/ Locality	No. examined	No. infected	Prevalence	95% exact binomial CI (%)
Khartoum (Khartoum and Jabal Aulya)	155	55	35.5	28-43.6
Um durman (Um durman, Karari and Um badah)	63	6	9.5	4.4-19.3
Khartoum North (Bahri and Sharq El Nile)	56	14	25	14.4-38.4
Total	274	75	27.4	22.2- 33.1

Table 3.2: Association between some risk factors and prevalence of equine piroplasmosis in horses in Khartoum State

Factor		**No. examined**	**Positive (%)**	**x^2**	**df**	***P*-value**
Sex	Stallion	201	45 (22.4)	9.428	1	0.002
	Mare	73	30 (41.1)			
Age	1-3	23	5 (21.7)	7.260	2	0.027
	(>3)-5	52	22 (42.3)			
	>5	199	48 (24.1)			
Breed	Cross-bred	39	8 (20.5)	1.076	1	0.300
	Local	235	67 (28.5)			
Body condition score	Thin	13	4 (30.8)	0.079	1	0.778
	Good	261	71 (27.2)			
Horse use (Activity)	Riding	169	32 (18.9)	16.949	2	0.000
	Sport	94	40 (42.6)			
	Other	11	3 (27.3)			
Housing system	Open yard	101	24 (23.8)	1.049	1	0.306
	Separate stable	173	51 (29.5)			
Previous infection (clinical signs)	Yes	22	8 (36.4)	0.973	1	0.324
	No	252	67 (26.6)			
Previous treatment	No	233	55 (23.6)	11.115	1	0.001
	Yes	41	20 (48.8)			
Tick infestation	Yes	123	26 (21.1)	4.363	1	0.037
	No	151	49 (32.5)			
Acaricide use	No	13	0 (0)	5.144	1	0.023
	Yes	261	75 (28.7)			
	Yes	39	10 (25.6)	0.069	1	0.793

Contact with other host species	No	235	65 (27.7)			
Biological control	No	257	74 (28.8)	4.210	1	0.040
	Yes	17	1 (5.9)			
Season	Summer	97	24 (24.7)	8.268	2	0.016
	Fall	66	27 (40.9)			
	Winter	111	24 (21.6)			

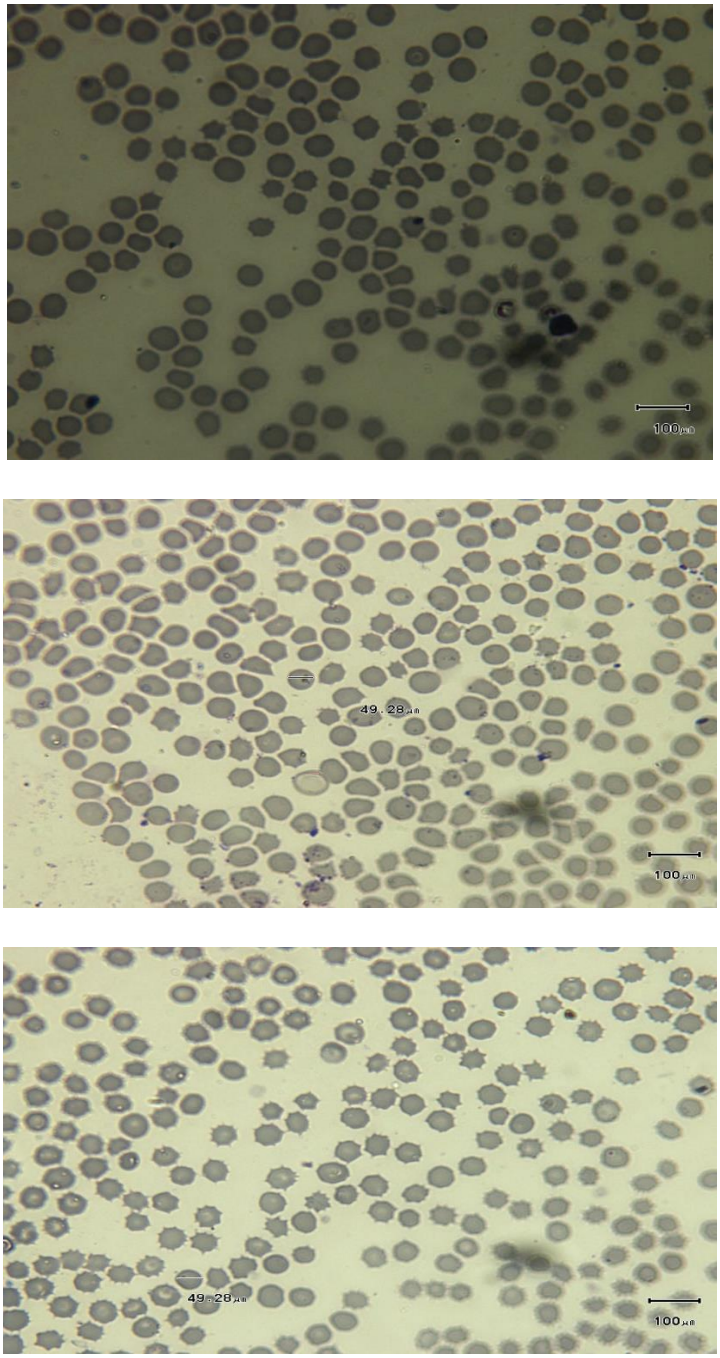

Figure 3.1: Positive Giemsa's stain for equine piroplasmosis

CHAPTER FOUR

DISCUSSION

The current cross-sectional study was conducted in the period from October 2016 to July 2017 to investigate the prevalence and risk factors for equine piroplasmosis in horses in Khartoum State using Giemsa stained blood smears. According to our survey, equine piroplasmosis was prevalent in all localities within Khartoum State with an overall prevalence of 27.4% (75/274). This prevalence was higher than that reported by Nada (2010) who found a prevalence of 8.3% in donkeys in Khartoum State by Giemsa staining technique. Our result was lower than that of a previous research in equine piroplasmosis in the Sudan by means of PCR amplification of the 18S rRNA gene of both *Thelieria equi* and *Babesia caballi*. In this investigation, Salim *et al*., (2013) stated a prevalence of 35.95% in Khartoum State. Prevalence of equine piroplasmosis in other countries has been reported to be 1.75% in working donkeys in Ethiopia (Mekibib *et al*., 2009), 41.6% in Egypt (Salib *et al*., 2012), 81.2% in donkeys examined by serology in Kenya (Oduori *et al*., 2015), 14% in Nigeria (Oladipo *et al*., 2015), molecular prevalence of 27.1% in Jordanian equids and 7.5% in pure Spanish breed horses tested serologically in Valencian community (Vega *et al*., 2012). These differences could possibly be attributed to difference in geographic locations, production system, diagnostic tests and healthcare like ticks control.

According to our observations, prevalence of equine piroplasmosis in mares 41.1% (30/73) was higher than that of stallions 22.4% (45/201) with significant association between sex and prevalence of equine piroplasmosis ($x^2 = 9.428$, $P = 0.002$). This result could be due to variation in sample size and also could be due to horse use as mares always kept for breeding purposes which expose them to excessive stress that weakens their immune system. This contradicts results detected by Turaki *et al.,* (2014) who investigated the prevalence of equine piroplasmosis amongst local horses in Northeastern Nigeria by Giemsa stain and found that the prevalence of equine piroplasmosis was higher in male horses than female horses. On the other hand, Liv (2009) reported that there was no statistically significant difference of the seroprevalence of equine piroplasmosis between male and female horses in the Swiss horse population.

The present survey considered age as a risk factor for equine piroplasmosis in horses ($x^2 = 7.260$, $P = 0.027$) with highest prevalence 42.3% and 24.1% recorded in both older age groups (>3)-5 and >5 years old, respectively whereas the lowest prevalence 21.7% found in 1-3 years old ones. This agrees with report of Kamyingkird *et al*., (2014). This finding could be explained by the fact that horses with middle and older ages were exposed to causative agents and vectors longer than younger animals. In contrast, Abedi *et al.,* (2013) stated that the prevalence of antibodies against *T. equi* in different groups of ages was not statistically different.

Regarding breed of animal, no significant association between equine piroplasmosis and breed was obtained ($x^2 = 1.076$, $P = 0.3$). However, the prevalence of equine piroplasmosis was higher in local breed (28.5%) compared with cross-bred horses (20.5%). This result could be due to difference in sample size between local breeds and cross-bred horses. This is similar to the work of Oladipo *et al*., (2015) who reported that prevalence of equine piroplasmosis in Nigeria was higher in local breed than foreign breeds.

Prevalence of equine piroplasmosis was higher in thin horses 30.8% (4/13) than horses with good body condition 27.2% (71/261) with no association between equine piroplasmosis and body condition score according to Chi-squared test ($x^2 = 0.079$, $P = 0.778$). Previous investigations of Liv (2009) in horses in Switzerland and Hawkins *et al.,* (2015) in grevy's zebras and donkeys in Kenya confirmed that there was no association between equine piroplasmosis and body condition score.

Our results showed significant differences in the prevalence of equine piroplasmosis among various horse activities ($x^2 = 16.949$, $P = 0.000$). Racing and jumping horses (sport horses) were approximately being infected with equine piroplasmosis two times greater than horses used in riding and more than one time greater than dragging and reproductive horses. This finding could be elucidated to the various degree of physical stress; in fact, many sport horses are exposed to intensive exercises that may considered as a stress factor and consequently immunosuppression. In our survey, the participated riding horses are given period of rest after patrolling (personal data). Similar results were recorded from Iran (Bahrami *et al*., 2014).

No significant association was observed between housing system and equine piroplasmosis ($x^2 = 1.049$, $P = 0.306$). However, the prevalence of the disease was

higher in horses kept in separate stables 29.5% (51/173) than those raised in open yards 23.8% (24/101). This result could be due to the possibility that separate stables could provide better conditions for ticks' survival and reproduction than open yard.

Equine piroplasmosis was more prevalent in animals that were subjected to Imidocarb treatment 48.8% (20/41) than animals that were not subjected to similar treatment 23.6% (55/233). In addition, Chi-squared test revealed strong association between equine piroplasmosis and previous treatment (x^2 = 11.115, *P* = 0.001). A possible explanation for this difference could be due to the fact that some clinical cases particularly with *T. equi* infection result in chronicity or carrier state after treatment course.

With regard to tick infestation, prevalence of equine piroplasmosis was 21.1% in horses that were infested by ticks at the time of sampling while it increases to 32.5% in horses that were not exposed to ticks. Chi-squared test showed that there was significant difference in the prevalence of equine piroplasmosis between the two categories (x^2 = 4.363, *P* = 0.037). This contradicted result could be due to the fact that manty owners used to apply tick control program after disease attack, but some cases continue as carriers despite that the program of control decreases vectors population.

In stables that used biological control of ticks (chickens), prevalence of equine piroplasmosis was lower 5.9% (1/17) than that in other stables without chickens 28.8% (74/257). Chi-squared test revealed significant association between equine piroplasmosis and biological control of ticks (x^2 = 4.210, *P* = 0.040), this result reveals the efficacy of biological control of ticks by chickens; however, it should be alongside with other control practices. Dreyer *et al*., (1997) reported that chickens are natural predators of ticks and they can be used as part of an integrated tick control plan.

Prevalence of equine piroplasmosis was higher during rainy season 40.9% (27/66) than summer 24.7% (24/97) and winter 21.6% (24/111) seasons. Chi-squared test showed significant association between disease and season (x^2 = 8.268, *P* = 0.016). This difference could be due to variation in ticks' number and transmission dynamic during different seasons of the year. Rüegg *et al.*, (2006) showed that the prevalence of equine piroplasmosis was 20% in spring and 100% in autumn.

Conclusion

It is concluded that equine piroplasmosis is endemic in Khartoum State and this could be attributed to many factors including age and sex of the horse, horse activity, biological control of ticks and season of the year.

Recommendation

1. Additional epidemiological studies using molecular techniques should be carried out to assess the exact prevalence of equine piroplasmosis.
2. Effective control program should be based on tick control and elimination of disease from carrier horses in addition to other factors.

REFRENCES

Abedi, V., Razmi, G., Seifi, H. and Naghibi, A. (2013). Seroprevalence of *Theileria equi* and *Babesia caballi* infection in Turkoman breed horses in Iran. Iranian J. Vet. Sci. Tech, 5:45-52.

Al-Obaidi, Q.T., Arshad M.M., Al-sultan, I.I., Azlinda, A.B. and Mohd Azam, K.G.K. (2016). Comparison between microscopic examination and competitive ELISA for diagnosis of equine piroplasmosis in Kelantan, Malaysia. Malaysian J. Vet. Res, 7:23-29.

Bahrami, S., Ghadrdan, A.R., Borujeni, M.P., Salarpur, M.V. (2014). Epidemiology of *Theileria equi* in Persian Arab horses from Iran. Vet. Med, 59:409-414.

Dreyer, K. L. J., Fourie, L. J. and Kok, D. J. (1997). Predation of livestock ticks by chickens as a tick-control method in a resource-poor urban environment. Onderstepoort J. Vet. Res, 64:273-276.

Hawkins, E., Kock, R., McKeever, D., Gakuya, F., Musyoki, C., Skilton, S.A., Njahira, M.N., Wamalwa, M. and Mania, E. (2015). Prevalence of *Theileria equi* and *Babesia caballi* as well as the identification of associated ticks in sympatric Grevy's zebras (*Equus grevyi*) and donkeys (*Equus africanus asinus*) in Northern Kenya. J. Wil. Dis, 51:137-147.

Henneke, D. R., Potter, G. D., Kreider, J. L. and Yeates, B. F. (1983). Relationship between condition scores, physical measurements and body fat percentage in mares. Equine vet. J., 15:371-372.

Janina, K. (2008). Effects of specific equine babesiosis treatments on equine oro-caecal transit time as measured by the lactose ^{13}C-ureide breath test. Ph.D. Thesis, Ludwig-Maximilians-University Munich. Germany.

Javed, K., Ijaz, M., Ali, M.M., Khan, I., Mehmood, K. and Ali, S. (2014). Prevalence and hematology of tick borne hemoparasitic diseases in equines in and around Lahore. Pakistan J. Zool, 46:401-408.

Kamyingkird, K., Yangtara, S., Desquesnes, M., Cao, S., Adjou Moumouni, P.K., Jittapalapong, S., Nimsupan, B., Terkawi, M.A., Masatani, T., Nishikawa, Y., Igarashi, I. and Xuan, X. (2014). Seroprevalence of *Babesia caballi* and *Theileria equi* in horses and mules from Northern Thailand. J. Paotozool. Res, 24:11-17.

Kerber, C.E., Labruna, M.B., Ferreira, F., Waal, D.T., Knowles, D.P. and Gennari S.M. (2009). Prevalence of equine Piroplasmosis and its association with tick infestation in the State of São Paulo, Brazil. Rev. Bras. Parasitol. Vet. Jaboticabal, 18:1-8.

Leticia, E. B. D.P., Roberto, N., Vincenzo, V., Francesca, I., Antonella, C., Luca, A.G., Francesco, F. and Teresa, S.M. (2016). *Babesia caballi* and *Theileria equi* infections in horses in Central-Southern Italy: Sero-molecular survey and associated risk factors. Ticks and Tick-borne Dis, 7:462-469.

Liv, U. Sigg (2009). Seroprevalence of equine piroplasmosis in the Swiss horse population. Ph.D. thesis. Vetsuisse-Fakultät der Universität Bern.

Mekibib, B., Manegerew, M., Tadesse, A., Abuna, F., Megersa, B., Regassa, A., Mekuria, S. and Abebe, R. (2009). Prevalence of haemoparasites and associated risk factors in working donkeys in Adigudem and Kwiha districts of Tigray region, Northern Ethiopia. The Int. J Vet. Med, 8:1-8.

Michael, M. (2016). Epidemiology of canine and equine piroplasmosis and their vector in Europe. Master's dissertation. Ghent University. Faculty of Veterinary Medicine. Belgium.

Nada, M.H. M. (2010). Prevalence of babesiosis in donkeys in Khartoum State, Sudan. Master of Tropical Animal Health (M.T.A.H.) thesis, Department of Preventive Medicine and Veterinary Public Health, College of Veterinary Medicine, University of Khartoum.

Oduori, D.O., Onyango, S.C., Kimari, J.N. and Macleod, E.T. (2015). A field survey for the seroprevalence of *Theileria equi* and *Babesia caballi* in donkeys from Nuu Division, Kenya. Ticks and Tick-borne Dis, 6:683-688.

Office International des Epizooties (OIE) (2009). Equine piroplasmosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals,6th ed. Office International des Epizooties, Paris. France.

Office International des Epizooties (OIE) (2014). Equine piroplasmosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Office International des Epizooties, Paris. France.

Oladipo, T.A., Adekunle, O.F. Okuneye O.J., Adebayo, M.D., Adeoye, A.T. and Banjoko, O.J. (2015). Pre and post polo competition of equine babesiosis in stable horses in Ibadan, Oyo State, Nigeria. International J. Res. Agri. For, 2:6-9.

Rùegg, S. (2008). Biology and Epidemiology of equine piroplasmosis in Takhin Tal (Mongolia). Ph.D. Thesis. Institute of Parasitology, Vetsuisse Faculty of Zurich, University of Zurich.

Rùegg, S. R., Torgerson, P. R., Doherr, m., Deplazes, P., Robert, N. and Walzer, C. (2006). Equine piroplasmosis at the reintroduction site of the Przewalski's horse (*Equus ferus przewwalski's*) in Takhin Tal, Mongolia. Doctoral thesis, University of Berne, Berne, Switzerland.

Salib, F.A., Youssef, R.R., Rizk, L.G. and Said, S.F. (2012). Epidemiology, diagnosis and therapy of *Theileria equin* infection in Giza, Egypt. Vet. World, 6:76-82.

Salim, B., Bakheit, M.A., Kamau, J. and Sugimoto, C. (2013). Current status of equine piroplasmosis in the Sudan. Infection, Genetics and Evolution, 16:191-199.

Soulsby, E.J.L (1982). Heminths, Arthropods and Protozoans of Domesticated Animals. 7th ed. London ELBS Tindall.

Thrusfield, M.V., 2007. Veterinary Epidemiology. 3rd ed. Oxford, Blackwell.

Turaki, U.A., Kumsha, H.A., Biu, A, A, and Bokko, P.B. (2014). Prevalence of piroplasmosis amongst local horses in Northeastern Nigeria. IOSR J. Agri. Vet. Sci, 7:4-7.

Vega, S., Domingo, R. (2012). Study of the Serological Prevalence of *Theileria equi* and *Babesia caballi* in Pure Spanish Breed Horses in the Valencian Community. Bulletin USAMV, 69:1-2.

Appendix I

Questionnaire

Date: …… Block, Locality and Location: …….....

Horse ID: ……...

Owner's Name: …….

Address and Telephone Number: …….

Age (year): ……

Sex:

Male ☐ Female ☐

Breed:

Local ☐ Foreign ☐ Crossbred ☐

Body Condition: ……

Horse Use:

Sport ☐ Riding ☐ Other ☐ Specify: …..

Housing System:

Separate Stable ☐ Open Yard ☐

Use of Acaricide:

Yes ☐ Specify: ……. No ☐

Biological Control:

Yes ☐ Specify: ……. No ☐

Tick Infestation:

Yes ☐ No ☐

Contact to other host species:

Yes ☐ Specify: ……. No ☐

Previous Infection (clinical signs):

Yes ☐ No ☐

Previous Treatment:

Yes ☐ Specify: ……. No ☐

Season:

Fall ☐ Summer ☐ Winter ☐

Giemsa stain:

Positive ☐ Negative ☐