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Surveillance of Theileriosis in Selected Dairy Farms in Khartoum and Gaziera States-Sudan

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Abstract: This study was conducted to investigate theileriosis in selected dairy farms in Khartoum and Gaziera States. One hundred and twenty blood samples were collected from six farms. From collected blood samples, smears were prepared and stained with Geimsa to demonstrate the piroplasm. The overall percentage of the samples that showed piroplasm was 24 20% distributed throughout the farms examined. Eighty four sera were prepared from the blood samples and examined by Enzyme Linked Immunosorbent Assay (ELISA) to demonstrate antibodies to *Theileria* Fifty four 64.3% samples demonstrated antibodies to *Theileria*. distributed throughout the farms examined. Ticks were collected from animals in different farms. All collected ticks were found to be belonged to the genus *Hyalomma* and species *antolicum. antolicum*

Key words: Theileriosis, piroplasm, demonstrate, ELISA

INTRODUCTION

Theilerioses is term that described a group of tick borne diseases affect domestic and wild ruminants. The disease is caused by members of genus *Theileria*[1].

The disease is characterized by clinical symptoms such as rise in body temperature, enlargement of lymph nodes namely parotid, prescapular and precrural[2]. Difficult breathing and frothy exudates from nostrils are reported[3].

Diagnosis of the disease is the first step for control[4]. Several diagnostic procedures have been described to diagnose the infection by *Theileria*[5,6]. The tentative diagnosis depends upon symptoms. Detection of the parasite can be achieved by preparation of blood smear that stained by Giemsa[6,7]. Microscopic examination shows *Theileria* schizanin in the lymph node and piroplasms in red blood cells. The morphological characteristics of the parasite may help to distinguish between the species[8,6].

Enzyme linked immunosorbent assay (ELISA) has been applied for serodiagnosis of infections with *T. annulata*[9] and *T. Parva*[10]. Different types of ELISA based on different antigens have been developed[3,11,12]. ELISA proved to be more sensitive and rapid than blood smear in the detection of theilerosis. The objective of the study was to investigate the disease in selected bovine farms and to compare ELISA and blood smear for detection of the infection.

MATERIALS AND METHODS

Study area and animals: The study was conducted in five dairy farms in Khartoum State namely, (Shambat, Koko, Geraif, Bagair) and one farm in Gaziera State (Geneid). The animals examined were divided in two groups: Group I animals are of four years old or above and Group II animals are calves less than one year old. The animals are of cross breed (Frisian X local) as well as local breeds (Kenana and Butana).

Samples: A total of 120 blood samples were collected from the six farms (20 from each farm). The samples were collected by vaccutainers directly from the jugular vein. Each sample was transferred into two containers, one with EDTA and the second was plain. The serum was separated by centrifugation at 1500 r m^{-1} for 10 min.

Blood smears: Thin and thick blood smears from all samples were prepared and stained with Geimsa to demonstrate the piroplasm.

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Enzyme Linked Immunosorbent Assay (ELISA): Eighty four serum samples (14 from each farm) were examined by ELISA to demonstrate antibodies to *Theileria*.

Indirect ELISA technique was used in this study. The working volume of each reagent was 100 μL. The plate wells were coated with 100 μL of 1/500 of the antigen in carbonate/bicarbonate buffer pH 9.6 and incubated at 37°C for one hour and then overnight in the refrigerator till used. Then washed 4 times with washing buffer containing 0.05% (v/v) Tween 20 in PBS. Blocking was done using 2% BSA fraction for one hour at 37°C. Then washed 4 times and prediluted 100 μL each of both positive and negative sera as controls were incorporated. The test sera were diluted 1/200 and added in duplicate and incubated at 37°C for one h. The plate was then washed four times and 100 μL of 1/500 rabbit anti-bovine peroxidse conjugated immunoglobulin (IgG) were added and incubated at 37°C for one h. The plate was then washed 4 times. and 100 μL of 2,2-azino-bis [3-ethylbenzthiazoline-6- sulfonic acid] and 2 mM hydrogen peroxide in 0.05 M citrate buffer pH 4.0 substrate were added and incubated at 37°C for 30 min. The results were read with the aid of spectrophotometer (Titretek Multiskan Plus, UK) using 405 nanometers wavelength.

Ticks collection and identification: Ticks were collected from animals in different farms. After collection, ticks were put in tubes containing 70% ethyl alcohol and sent to laboratory for identification. Ticks identification was done based on macro and microscopical morphology according to Hoogstraal[13].

RESULTS

Out of 120 blood smears prepared, 24 (20%) showed piroplasm stage. The distribution of the positive samples through the farms was as follow :Koko farm, 2 (10%); East Gereif farm, 1 (5%); Bagair, 17 (85%); Geneid local farm, 4 (20%). The distribution of the positive and negative samples is shown on Table 1.

Out of the 84 serum samples tested by ELISA, 54 (64.3%) demonstrated antibodies to *Theliera*. The distribution of the positive samples through the farms was as follow: Shambat, 10(71.4%); Koko farm, 13 (92.9%); East Gereif farm, 11 (78.6%,); Bagair, 7 (50%); Geneid local farm, 4 (28.6%); Geneid sugar factory farm, 9 (64.3%). The distribution of the positive and negative samples is shown in Table 2.

Group I animals (more than four years old) showed high infectivity than group II (less than one year old). All collected ticks were found to be belonged to the genus *Hyalomma* and species *antolicum antolicum*.

Table1: Distribution of positive and negative blood smears

Farms	No. of samples tested	No. of positive samples (%)	No. of negative samples (%)
East geraif	20	1 (5%)	19 (95%)
Koko	20	2 (10%)	18 (90%)
Geneid local	20	4 (20%)	16 (80%)
Bagair	20	17 85%	3 (15%)
Shambat	20	0 (0%)	20 (100%)
Geneid sugar factory	20	0 (100%)	20 (100%)
Total	120	24 (20%)	96 (80%)

Table 2: Distribution of positive and negative serum samples

Farms	No. of samples tested	No. of positive samples (%)	No. of negative samples (%)
Geneid local	14	4 (28.6%)	10 (71.4%)
Bagair	14	7 (50.0%)	7 (50.0%)
Geneid sugar factory	14	9 (64.3%)	5 (35.7%)
Shambat	14	10 (71.4%)	4 (28.6%)
East gereif farm	14	11 (78.6%)	3 (21.4%)
Koko	14	13 (92.9%)	1 (7.1%)
Total	84	54 (64.3%)	96 (35.7%)

DISCUSSION

Theileriosis is one of important parasitic diseases in Sudan that have great economical impact[14]. Usually local breeds have tolerance to infection while exotic and cross breeds are highly susceptible and great loss due to the disease do occur. This study was conducted in different farms in Khartoum and Gaziera States in Sudan to determine the presence of theileriosis based on blood smears and serology and to evaluate the two tests in detection of the infection.

The prevalence in all farms based on blood smears was 20% while the prevalence based on ELISA was 64.3% which considered very high. This may proved the high sensitivity of the test. The low prevalence based on smears may be attributed to the some infected animals which demonstrated antibodies passed the parasitaemia and the organism is no longer found in the blood.

Geneid sugar factory farm showed low prevalence by two tests. This probably may be due to the fact that this farm practiced ticks control as well as routine examination. Although ELISA proved to be more sensitive than blood smear but its routine use in farms is difficult due to the high cost and sophistication of the test, on the other hand blood smear is quick and cheaper[15]. It could be concluded that theilerosis is a serious problem in examined farm and ELISA is a good test for detection of the infection compared to blood smear.

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