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Journal of Veterinary Medicine and Research

Case Report

Theileria spp. in Free Ranging Giraffes (*Giraffa camelopardalis*) in Zambia

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Submitted: 28 November 2015

Accepted: 30 December 2015

Published: 31 December 2015

ISSN: 2378-931X

Keywords
- Game ranch
- Giraffe
- *Giraffa camelopardalis*
- Piroplasms
- *Theileria*
- Ticks
- Wildlife

Abstract

Theileria parasites were detected in five apparently healthy free-ranging giraffes (*Giraffa camelopardalis* Linnaeus, 1758) captured for translocation on a game ranch located approximately 60 km south west of Lusaka. Giemsa-stained blood smears examined under a light microscope showed characteristic oval and rod shaped intra-erythrocytic piroplasms. Polymerase chain reaction (PCR) products targeted on the 18S rRNA gene showed characteristic bands of *Theileria* spp. The average number of infected blood cells per field examined by light microscopy was estimated at 48.6% (n=50, SD±8.2%). The mean white blood cell count (WBC), red blood cell count (RBC), haemaglobin and packed cell volume (PCV)(%) for the five giraffes were estimated at 8.0 x 10^3/µl, 7.9 x 10^6/µl, 17.8 g/dL and 41.8%, respectively, being within the normal range of hematological values of free-ranging healthy giraffes. Tick species collected from the infected animals included *Rhipicephalus appendiculatus*, *Rhipicephalus* spp., *Amblyomma variegatum* and other *Amblyomma* species. To our knowledge, this is the first report of *Theileria* spp infection in giraffe in Zambia. These findings suggest that wildlife kept on game ranches could serve as carriers of *Theileria* piroplasms without expressing clinical signs of theileriosis even when infected with a high parasitemia. As such, it is likely that these wildlife reservoirs could play an important role on the epidemiology of theileriosis in Zambia, although detailed follow-up studies are required to determine the intra- and interspecies transmission among wildlife as well as between wildlife and livestock.

INTRODUCTION

There has been an increase in the number of wild animals kept in captivity for ecotourism and conservation in Zambia. Consequently, this has heightened the interest in the disease spectrum and prevalence in wildlife given the close proximity to livestock rearing areas and general concerns by veterinary authorities that wildlife are reservoir hosts of economic importance. Therefore, as more research is carried out, disease is increasingly being recognized as a major threat not only to wildlife conservation but to the livestock sub sector as well [1]. Thus, with the rapid expansion of the game ranching industry, translocation of various species to game ranches may likely ignite an unknown array of various disease outbreaks originally naïve to livestock, which could pose a significant threat to the livestock industry in the absence of robust veterinary disease control services. Among these diseases is theileriosis that has had a devastating effect to the livestock industry in Zambia whose reservoir hosts include wildlife species such as the African buffalo (*Syncerus caffer*).

Theileriosis is an intracellular parasitic disease that is predominantly widespread in the tropical and sub-tropical regions. However, the *Theileria* species of domestic cattle probably originated from wild bovidae that may still be the reservoir hosts with the African buffalo being considered as the natural host of *Theileria parva parva* and *T. p. lawrencei* [2]. In wild animals, *Theileria* infections are generally subclinical, whereas in cattle theileriosis takes a lethal course [3]. Several *Theileria* species have been described in wild animals and a single animal carrying multiple species has been described in domestic animals and wildlife [4-6]. In Southern Africa, *Theileria* species have been reported in Kudu *(Tragelaphus strepsiceros)* and Sable

antelope *(Hippotragus niger)*, common gray duiker *(Sylvicapra grimmia)*, roan antelope *(Hippotragus equinus)*, giraffe and Buffalo [7-10]. Although, different species of *Theileria* parasites have been isolated in Zambia [11-13], none of these parasites have been reported in giraffe (*Giraffa camelopardalis*). Hence, the objective of the present study was to test the five giraffes captured for translocation on a game ranch for the presence of blood parasites as an overture to increasing our understanding of the disease profile of giraffes reared on game ranches in Zambia. Based on the screen test carried out, we report for the first time the detection of *Theileria* spp blood parasites in apparently healthy free-ranging giraffes.

METHODS AND MATERIALS

Study Area

The study was carried out on a 1500 hectare game ranch located 60 km south of Lusaka on the banks of the Kafue river (S 15°54'50.46" E 28°27'71.46") in May 2012. The game ranch, which is located on the Kafue flats, has a typical savannah grassland vegetation with the most common grass species being the *Cynodon dactylon*, *Hyperrhenia* spp. and *Tristachia hispida* while the common tree species were *Accacia polyacatha* and *Albezia* spp. At the time of capturing the giraffes, the game ranch had a total population of 1834 wild animals from 20 different species, which included giraffe (*Giraffa camelopardalis*), wildebeest (*Connochaetes taurinus*), hartebeest (*Alcelaphus lichensteinii*), eland (*Taurotragus oryx*), zebra (*Equuus burchelli*), Kafue lechwe (*Kobus leche kafuensis*), kudu (*Tragelaphus strepsiceros*), duiker (*Sylvicapra grimmia*), impala (*Aepyceros melampus*) and puku (*Kobus vardonii*). There were no African buffaloes reared on the game ranch before and at the time of capturing the giraffes. The total number of giraffes on the game ranch at the time of capture was nine. These giraffe were introduced on the ranch from neighbouring Zimbabwe in the late 1980s.

Tick control on the game ranch was carried out using a sweeper herd of cattle, in which 210 cattle were allowed to graze and move around the game ranch freely. In this way, ticks found on the game ranch infested cattle as they grazed and then the infested cattle were treated with acaricides (Drastic Deadline® (Flumethrin 1% m/v) Pour-on, Acaricide, Bayer Animal Health, Paracide Plus® (Alphamethrin 7 % m/v) Zoetis, and Tick Grease® (Deltamethrin) Coopers) once a week during the rainy season or once every fortnight during the dry season. The principle idea is that cattle, which are easier to handle because they are tamed, are used to sweep off the tick population on the game ranch and by treating them using acaricides that kill the ticks, they are used to reduce the tick burden on the game ranch. However, records on the game ranch showed that cases of theileriosis in cattle had been diagnosed on the game ranch two years prior to the translocation of the giraffes used in this study. Infected cattle showed classical signs of cattle theileriosis leading to high mortality and all animals were treated with anti-theileriosis drugs to which they responded positively.

Capture of animals and sample collections

Animal capture: Five giraffes were immobilized using a combination of etorphine hydrochloride (M99®- Novatis, Johannesburg, South Africa), thiofentanil oxalate (A3080®-Wildlife Pharmaceuticals) and hyaluronidase (Hyalase -Kyron) delivered by remote injection to the animals on the game ranch. Thereafter, clinical examination was carried out to establish the health status of each animal. The general body condition of the animals was good and no clinical signs were observed for theileriosis or other diseases. The age and sex of each animal was determined and age was classified as adult, sub-adults or young. After clinical examination and sample collection, the animals were reversed using M5050 Revivon® (diprenorphine) at standard capture doses (Norvatis SA Ltd, Animal Health, Johannesburg, South Africa) and transported to their new habitat. All animals were captured under authorization of the Zambia Wildlife Authority (ZAWA/01/2012) the custodians of all wildlife in Zambia. During the capture process prior to translocation, one giraffe (designated as giraffe 1 in Table 1) died due to capture myopathy and no post-mortem examination was carried out. After four weeks when the animals had acclimatized at the new habitat they were observed for clinical signs to check whether they had succumbed due to stress induced by the capture process. All animals looked healthy and they had adapted well to their new habitat.

Blood sample collection: Blood samples were collected by venipuncture of the jugular vein in EDTA and plain vacutainer tubes. Thick and thin blood smears were made from stubs on the ear veins for the detection of blood parasites while whole blood in EDTA tubes was used for complete blood counts. Blood samples in EDTA tubes were stored at -20^{0}C until DNA extraction. Blood samples collected in plain vaccutaner tubes were stored at 4^{0}C overnight to allow blood clotting followed by centrifugation at 2500 rpm for 10 minutes to separate the serum from the blood clots. Sera was collected in cryogenic vials and stored at -20^{0}C.

Tick collection: Ticks were manually collected from the examined animals and stored in aerated vials for identification at the School of Veterinary of Medicine, University of Zambia in Lusaka. The identification of ticks was carried out using a standard key as described elsewhere [14]. After tick collection, all animals were treated with a pour-on acaricide (Drastic Deadline® (Flumethrin 1% m/v) Pour-on Acaricide, Bayer Animal Health, Paracide Plus® (Alphamethrin 7 % m/v) Zoetis, and Tick Grease®(Deltamethrin)- Coopers) to prevent the transfer of ticks to the new habitat after translocation.

DNA extraction: One microliter of saponin lysis buffer was added to 400µl of each blood sample and the mixture was vortexed. This mixture was centrifuged at 10,000 x g for three minutes. The supernatant was carefully removed and discarded. The pellet was re-suspended in 0.75ml lysis buffer, vortexed and centrifuged at 10,000 x g for three minutes. This step was repeated until the pellet was clear of any traces of haemoglobin. The pellet was then re-suspended into 50µl of 50Mm KCl, 10Mm Tris-HCl and Tween after which it was incubated at 56-C in a water bath for 2 hours. The extracted DNA was then diluted with nuclease free water at the ratio 1:4 and stored at -20°C until use. In the final step, genomic DNA was eluted in sterile deionized water at a final volume of 100uL per tube and each tube was stored at -20^{0}C until use.

Laboratory diagnosis

Blood smears: Examination of the blood smears on the slides

Table 1: Sex, age and tick species collected from the captured giraffes.

Giraffe Nº.	Gender and age	Tick species collected
1	Male, adult	*Rhipicephalus appendiculatus, Amblyomma variegatum, Amblyomma* spp.
2	Male, sub adult	*Rhipicephalus* spp. *A. variegatum, Amblyomma* spp
3	Male, sub adult	*R. appendiculatus, Rhipicephalus* spp. *Amblyomma* spp.
4	Female, sub adult	*R. appendiculatus, Rhipicephalus* spp. *Amblyomma* spp.
5	Female, sub adult	*R. appendiculatus, Rhipicephalus* spp.

Table 2: Haematological values of the captured giraffes.

Parameters	Giraffe 1	Giraffe 2	Giraffe 3	Giraffe 4	Mean	Reference Values [16]
WBC x 10^x/L	7.8	6.8	9.8	7.4	8.0	4.0-24.5
RBCs x 10^x/L	8.56	6.98	7.3	8.6	7.9	5.8-19.1
PCV	35	41	43	48	41.8	27.0-56.0
Plasma protein (g/dL)	6.8	7	7	7	6.95	6.2-10.5
Haemoglobin (g/dL)	14.8	18.2	18.4	19.6	17.8	9.4-19.7
MCV(fl)	40.8	58.7	58.9	55.8	53.6	21-57
MCHC(%)	42.2	44.3	42.7	40.8	42.5	29-40
MCH(pg)	17.2	26.1	25.2	22.7	22.8	7-20
Fibrinogen (g/dl)						
Band neutrophils	1	2	1	0	1	0.0-0.7
Segmented neutrophils	49	38	52	42	42.3	-
Lymphocytes	39	53	37	48	44.3	0.0-1.4
Eosinophils	7	3	6	7	5.8	0.0-1.4
Monocytes	4	4	4	3	3.8	0.0-1.3
Basophils	0	0	0	0	0	0-0-2.7

Giraffe five had no haematological values as the blood that was collected was severely haemolysed upon arrival at the lab and could not be analysed, Blood smear examination however, demonstrated the presence of intra-erythrocytic piroplasms.

was carried out using the Giemsa stain followed by observation under a light microscope. Briefly, blood smears were fixed in methanol for 5 minutes followed by immersion in Giemsa solution for 30 minutes. Thereafter, the slides were examined for blood parasites using a 100x lens under oil immersion. Approximately 100 fields were examined per slide. To estimate the level of parasitemia in the infected animals, the total number of blood cells observed per field was counted followed by counting the number of cells infected by the piroplasms, in ten randomly selected fields per animal. The proportion of infected was determined by dividing the number of infected cells with the total number of blood cells observed per field examined.

Polymerase chain reaction test: Amplification of PCR products was carried out using primers previously described by Georges et al as cited by Kursat et al [15] to amplify a *ca* 400 base pair (5'-GACACAGGGAGGTAGTGACAAG-3' and 5'-CTAAGAATTTCACCTCTGACAGT-3') DNA fragment of the 18S rRNA gene. PCR cycle conditions were as described Georges et al as cited by by Kursat et al [15]. All PCR products were visualized after electrophoresis in a 1.5 % agarose gel containing 0.2µg Medori Green by transillumination using UV light. DNA extracted from the five giraffes were used for this study including DNA extracted from a positive control sample obtained from *Theileria* used for experimental infection studies in cattle used at the School Veterinary Medicine, University of Zambia in Lusaka. The negative control sample was from DNA extracted from whole blood of a cow that was known and previously tested to be negative of theileriosis.

RESULTS

Overall, the general clinical condition of the animals was good. There was no swelling of lymph nodes and the mucus membranes were not pale with no other clinical signs observed. Blood parasites detected by Giemsa staining are shown as intracellular piroplasms (Figure 1). The parasites were mainly oval or rod in shaped as shown in Figure 1. Generally, the erythrocytic parasites showed a high parasitemia given that a large proportion of the red blood cells (RBC) were infected by the *Theileria*-like piroplasms as shown in Figure 1. This finding was consistent for all five giraffes. To estimate the level of parasitemia based on the number of infected cells per microscopic field, 50 fields were examined per animal and the average number of infected RBCs was estimated 48.6% (n=50, SD±8.2).

Generally, the number of ticks found on each animal was low and the tick species identified are shown in Table 1. Table 2 shows the haematological values detected for the five giraffes in comparison to values previously reported by other scientists for normal healthy giraffes [16-19]. Figure 1 shows products of

the five giraffes expressed at *ca* 400 base pair fragments detected by the polymerase chain reaction (PCR) test. All five giraffes including the positive control sample expressed the same band as shown in Figure 2 and only the PCR negative control had had no band detected.

DISCUSSION

Translocation of wildlife for conservation and ecotourism has tremendously increased in recent years because of the expanding game ranching industry in Zambia. This expansion has brought with it the need for screening wild animals that are captured for translocation for different diseases thereby increasing our knowledge of the disease spectrum of wildlife in Zambia [10,14,20-23]. Pre-translocation disease screening is an important disease surveillance strategy because it enables identification of diseases that would potentially serve as a source of infection to susceptible animals in the new habitats [24]. Once, these diseases are identified the captured animals can be treated or vaccinated prior to translocation in order to prevent the spread of wildlife diseases to naïve populations.

The giraffes examined in the present study did not show clinical signs for theileriosis and other diseases which is similar to observations made by Oosthuizen *et al* [8] in South Africa where *Theileria* piroplasms were detected in healthy free-ranging giraffes. In addition, the hematological parameters examined in the present study were within the normal range of those reported by other scientists [16-19] for healthy giraffes. These findings suggest that despite the high parasitemia with an infection rate of 48.6% (n=50, 8.2%) of the RBCs per microscopic field examined these blood parasites did not have adverse effects on the health status of the animals. Given that cattle that were used as a sweeper herd for tick control two years prior to the current study had an outbreak of theileriosis linked to high mortality, it is likely that the ticks found on the game ranch were carriers of *Theileria* parasites and that these ticks transmitted the disease to both wildlife and cattle on the game ranch. Although different wildlife species might have been infected by *Theileria* piroplasms on the game ranch, only cattle were able to express the clinical disease rendering wild animals to be reservoir hosts for the disease. This is in line with observations made Grootenhuis et [3] that cattle are susceptible hosts that develop clinical disease while wildlife serve as reservoir hosts that do not develop clinical disease. Given that other wild animals present on the game ranch at the time of translocation were not examined, it is not known whether the giraffes examined in this study played a major role as reservoir hosts of the disease that infected cattle used as sweeper herds for ticks control. Hence, to fully understand the role of giraffes and other wildlife species on the epidemiology of theileriosis in the area, there is a need for detailed follow-up studies aimed at determining the inter-species transmission of the disease between wildlife and cattle. However, it is important to point out that previous studies carried out by Purnell et al [25] and Grootenhuis et al [26] showed that transmission of *Theileria* parasites from impala and wildebeest to cattle failed although the parasites were transmitted between animals of the same species. Data obtained from these studies can be used to determine whether different wildlife species harbour *Theileria* spp. that cause clinical disease in cattle and to determine whether the use of cattle as sweeper herds for tick control is an effective disease control strategy.

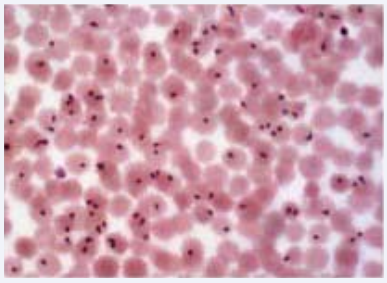

Figure 1 Light microscopy of Giemsa stained thin blood smears showing Theileria piroplasms in red blood cell in giraffe 3.

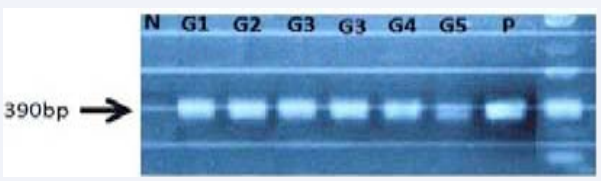

Figure 2 Results for the polymerase chain reaction (PCR) test for the five giraffes on DNA samples collected from whole blood. Lanes G1 to G5 shows the ca 390 base pair band of the DNA fragment of the 18S rRNA gene. Similarly, lane P ca 390 bp band for the positive control sample while lane N which was the negative control sample shows no band detected by PCR.

Thus far, five *Theileria* species or subspecies have reported from cattle in Zambia namely *Theileria p. parva*, *T. p. lawrenci*, *T. mutans*, *T. traurotragi*, and *T. verifera* [11-13]. There are no studies carried out this far showing the presence of these *Theileria* spp.. in wildlife in Zambia. Given that the tick species linked to *Theileria* transmission are widely distributed and that they have been collected from different wildlife species on game ranches, National Parks and game management areas [10,23,27] it is likely that different *Theileria* spp. alongside their transmitting vectors and wildlife reservoirs could be widely distributed in Zambia. Hence, we anticipate that the findings obtained from this study will stimulate the need for detailed investigations that will include molecular characterization of different *Theileria* spp. found in wildlife and further investigate the inter- and intra-species transmission of theileriosis in Zambia. Data emanating from such studies would help elucidate the role of theileriosis in the conservation of wildlife as well as improving our understanding of the epidemiology of theileriosis in Zambia.

ACKNOWLEDGEMENTS

The authors are grateful to the Zambia Wildlife Authority for permission to carry out this study.

Authors' contribution: KSN, SD, MM and HK= capture of animals, samples collection and preparation of manuscript. KSN, EM and HM laboratory analysis and preparation of the manuscript. All authors read and approved the final version of the manuscript.

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Cite this article

Nalubamba KS, David S, Munyeme M, Kamboyi H, Saasa N, et al. (2015) Theileria spp. in Free Ranging Giraffes (Giraffa camelopardalis) in Zambia. J Vet Med Res 2(5): 1039.