مجلة السّودان العالميّة للبحوث
INTERNATIONAL JOURNAL OF SUDAN RESEARCH

V11 • N2 • 2021 | 2021 • العدد 2 • المجلد 11

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تنشر بالتعاون مع المنظمة العالمية للتنمية المستدامة ومعهد الشرق الأوسط للاقتصاد المبني على المعرفة لندن، المملكة المتحدة.

رقم الإيداع بالمكتبة البريطانية
ISSN: 2042-6003 (PRINT), 2042-6011 (ONLINE)

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DETECTION OF BLOOD PARASITES AND TOXOPLASMA GONDII IN WILD ANIMALS IN DINDER NATIONAL PARK, SUDAN

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IJSR

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International journal of
SUDAN RESEARCH

ISSN: 2042-6003 (Print) | 2042-6011 (Online)

IJSR V11 N2 2021

OPEN ACCESS

DOI: 10.47556/J.IJSR.11.2.2021.4

RESEARCH PAPER

Detection of Blood Parasites and Toxoplasma gondii in Wild Animals in Dinder National Park, Sudan

Professor Aisha Elfaki Mohamed
Wildlife Research Center, Animal Resources Research Corporation
Ministry of Animal Resources, PO Box 16 Elmorada Omdurman
Khartoum, Sudan
Email: aishelfaki@hotmail.com

Dr Manal Yosif Ishag
Director of Academic Affairs and Training
Central Veterinary Research Laboratory
PO Box 8067 Alamarat
Khartoum, Sudan
E-mail: medanimanal@gmail.com

Dr Mohamed Siyab Eldin Ahmed
University of Khartoum
PO Box Khartoum North
13315 Shambat, Sudan
E-mail: m_sea_74@hotmail.com

CITATION: Mohamed, A., E., Ishag, M., Y., Ahmed, M., SE. (2021): Detection of Blood Parasites and Toxoplasma gondii in Wild Animals in Dinder National Park, Sudan, International Journal of Sudan Research, Vol 11, No. 2, pp. 157-170.

RECEIVED: 22 November 2020 / **REVISED:** 14 September 2021 / **ACCEPTED:** 15 September 2021 / **PUBLISHED:** 10 November 2021

Abstract:

Purpose: The study aims to investigate the presence of blood parasites and/or *Toxoplasma gondii* in wild animals at Dinder National Park, Sudan.

Design/methodology/approach: Wild animals, including two female warthogs (*Phacochoerus aethiopicus*) (2.5-4 years), two male reedbuck (*Redunca redunca*) (1-2.5 years), and one male water buck (*Kobus defassa*) (3.5-4 years) were darted. Health observations, clinical and clinico-chemical parameters were determined. Blood samples were collected immediately after anaesthesia of animals. Blood smears were made for each blood sample to detect blood parasites. Sera were harvested from the blood samples and ELISA was carried out for the detection of IgG antibodies targeted against *Toxoplasma gondii*. Whole blood samples were used for DNA extraction in order to evidence the presence of *T. gondii*. A real-time polymerase chain reaction (PCR) was applied to detect the B1 gene from purified DNA samples and some haematological parameters were determined.

Findings: Body temperature (ºC) was elevated in reedbuck (45.34±0.8) while the pulse rate of the reedbuck (Pulse/min.) 43.92±17.71 and respiratory rate of the warthog (Breath/min) 41.46±10.65 were also elevated. *Anaplasma spp.* was detected in one of the reedbuck and both warthogs blood smear samples, while *Theileria spp* was detected in the other reedbuck and water buck samples. Positive anti-*Toxoplasma* IgG antibodies were detected in the two warthog sera samples. *T. gondii* was detected from whole blood in only one warthog. The average levels for the haematological parameters were: WBC were $8.25\pm1.23x10^3/mm^3$, $2.45\pm0.95x10^3/mm^3$ and $3.8\pm0.72x10^3/mm^3$, RBC were $5.03\pm3.86x10^6/mm^3$, $7.52\pm1.22x10^6/mm^3$, and $11.27\pm1.66x10^6/mm^3$, and Hb were 12.75±1.69g/dl, 17.35±1.23g/dl and 16.87±2.86g/dl for the warthog (*Phacochoerus aethiopicus*), reedbuck (*Redunca redunca*), and water buck (*Kobus defassa*) respectively.

Originality/value: The findings of this study confirm that wildlife in Dinder National Park carry *Anaplasma, Theileria* and *T. gondii* organisms, and could serve as reservoirs of infection for domestic animals.

Research limitations/implications: Further studies are needed to establish solid findings on the blood parasites and *Toxoplasma gondii* in wild animals in Dinder National Park.

Practical implications: Detailed investigations, including molecular characterisation of different *Theileria spp*. and *Anaplasma spp*., and further investigation of the inter- and intra-species transmission of *theileriosis* in wild animals at Dinder National Park are needed.

Paper type: Research paper

Keywords: *Wild animals, Toxoplasma gondii, Theileria spp, Anaplasma spp, Dinder National Park, Sudan.*

Introduction

The mixing of wild animals into one habitat is likely to be a proponent of introducing diseases and otherwise exposing animals to parasitic infections. Concern about the transmission of infectious agents between wildlife and domestic livestock is increasing, especially in areas where free-ranging wildlife and cattle share common grazing grounds (Kocan *et al.,* 2010). *Theileriosis* in wildlife has been described in antelopes (Nijhof *et al*., 2005). Several *Theileria* species have been described in wild animals, and a single animal carrying multiple species has been described in domestic animals and wildlife (Oura *et al*., 2011; Bazarusanga *et al*., 2007). 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 (Pfitzer *et al*., 2011). Among African wildlife, the sub-clinical occurrence of *Anaplasma marginale*, either natural or after artificial infection, has been confirmed in the African buffalo (*Syncerus caffer*) (Potgieter, 1979), *Taurotragus oryx* (Ngeranwa, *et al*., 1998), black wildebeest (*Connochaetes gnou)* (Neitz, 1935), blue wildebeest (*Connochaetes taurinus*) (Smith *et al*., 1974), grey duiker (*Sylvicapra grimmia*) (Neitz and Du Toit, 1932) and blesbok (*Damaliscus dorcas phillipsi*) (Neitz and Du Toit, 1932). *Anaplasma marginale* was successfully transmitted from a naturally infected giant African rat (*Cricetomys gambianus*) to a bovine (Dipeolu *et al*., 1981).

Toxoplasma gondii is an intracellular protozoan parasite that has a heteroxenous life cycle and infects all warm blooded animals (pets, wild and farm animals) and humans (Acha and Szyfres, 2003; Cenci-Goga *et al*., 2011). Wild and domestic felids are the definitive hosts; these release parasite oocysts in their faeces (Tenter *et al*., 2000). Other mammals, including wild game species, act as intermediate hosts that can become infected by ingesting oocysts or parasitised tissues. da Silva *et al*. (2014) reported that *T. gondii* antibodies were detected in 6 of 26 run over or injured wild animals treated at the Veterinary Hospital of University Centre of Rio Preto (UNIRP), Brazil. Acute and potentially fatal infections were recorded in New World monkeys (Cunningham *et al*., 1992), marsupials (Canfield *et al*., 1990) and certain other animals. De Craeye *et al*. (2011) tested *cervidae* sera for the presence of antibodies against *Toxoplasma gondii* using SAG1-ELISA and a commercially available agglutination test. They found that the *T. gondii* sero-prevalence was 52% in roe deer (*Capreolus capreolus*), 0% in bred fallow deer (*Dama dama*), red deer (*Cervus elaphus*), and 18.8% in red foxes.

Materials and Methods

Study area:

This study covered potential sites in the oldest natural park in northern Sudan, Dinder National Park, Sudan. The park has an area of 1.084.6 00 hectares and is located in the southern part of the central region, adjacent to the Ethiopian border with the Blue Nile Province (Dasmann, 1972). The park falls within the Savannah zone, which is characterised by a long summer (April-October). From November to February there is cool dry weather with daily maximum temperature averaging 30ºC (86ºF). Peak rains are during August with a minimum of 600mm and a maximum of 1,000mm (Awad, 1985). Harrison and Jackson (1958), Holsworth (1968), Dasmann (1972), Elsammani (1982) and Hashim (1984) described the geomorphology and vegetation in the Dinder National Park. The park is an island of a diverse array of fauna and flora of the region, and the soil is a dark coloured cracking clay. Hakim *et al*. (1978) recognised three types of ecosystem: *A. seyal*, *Balanites aegyptiaca* savanna woodlands; a riverine ecosystem dominated by *Hyphaene thebaica*, *Acacia siberiana, Stereospermum kukthianum, Entada sudanica, Ficus sycomrous, Combretum spp. and Tamarindus indica*.; and the Wetlands (known locally as mayas) ecosystem dominated by mat-shaped vegetation, e.g., *Cynadon dactylon, Typha sp., Ipomaea aquatic* and *Kyllinga sp.* The most striking feature of the park is the presence of these wetlands (mayas), important because they are a source of water. Dinder National Park provides the habitat for about 27 types of large mammals. According to Dasmann (1972), the animals remain in the park during the dry season from November to May and several species leave at the onset of the rains in June.

Animal study:
Blood samples and smears were collected from two female warthogs (*Phacochoerus aethiopicus*) (2.5-4 years old), two male reedbuck (*Redunca redunca*) (1-2.5 years old), and one male water buck (*Kobus defassa*) (3.5-4 years old) during a training immobilising course in Dinder National Park, Sudan. The animals were immobilised using M99 (etorphine hydrochloride, Norvatis Ltd, Johannesburg, South Africa) at standard doses. All animals were captured under authorisation of the Wildlife Conservation General Administration, Sudan. Thereafter, clinical examination was carried out to establish the health status of each animal: body temperature, pulse rate and respiration were recorded. After clinical examination and sample collection, the animals' immobilisation was reversed using M5050 Revivon® (diprenorphine) at standard capture doses (Norvatis SA Ltd, Animal Health, Johannesburg, South Africa).

Sample collection:
Blood samples were collected by venepuncture of the jugular vein in each animal, heparinised and placed in plain vacutainer tubes. Thick and thin blood smears were made for the detection of blood parasites while the whole anti-coagulated blood was used for the determination of total erythrocyte count, haemoglobin (Hb) concentration, and total leukocyte count according to the method of Dacie and Lewis (1991). For blood samples that were collected in plain vacutainer tubes, centrifugation was applied at 4,000rpm for 10 minutes at room temperature to separate sera. Sera were collected in cryogenic vials and stored at -20°C. Whole blood samples were used for DNA extraction in order to evidence the presence of *T. gondii*.

Microscopic examination:
Examination of the blood smears on the slides was carried out using Giemsa stain, followed by observation under a light microscope.

Serological analysis:
An immuno-enzymatic test (ELISA) was carried out for the detection of IgG antibodies targeted against *T. gondii* according to the manufacturer's instructions (DRG Instruments GmbH, Germany).

Molecular analysis:
DNA was extracted from the leukocyte fraction of the whole blood with a commercial kit using the Illustra Tissue and Cells Genomic Prep Mini Spin Kit (GE Healthcare Life Sciences do Brasil Ltda®, Brazil). The target DNA for real-time quantitative polymerase chain reaction (PCR) amplification was the published sequence of the 35-fold repetitive B1 gene of the *T. gondii* RH strain. Template DNA was added to a reaction mixture containing 25µl of 2× PCR universal master mix, 5µl of the forward primer TOXO-F (5µM, 5′-TCCCCTCTGCTGGCGAAAAGT-3′), 5µl of the reverse primer TOXO-R (5µM, 5′-AGCGTTCGTGGTCAACTATCGATTG-3′), and 5µl of Taq Man probe (2µM, 6FAM-TCTGTGCAACTTTGGTGTATTCGCAG-TAMRA) in a final volume of 50µl. The PCRs were performed with the Gen Amp 5700 Sequence Detection System (PE Applied Biosystem). After initial activation of AmpliTaq Gold DNA polymerase at 95°C for 10 minutes, 40 PCR cycles of 95°C for 15 seconds and 60°C for 1 minute were performed. The cycle threshold value (C_T), indicative of the quantity of the target gene at which the fluorescence exceeds a pre-set threshold, was determined. This threshold was defined as 20 times the standard deviation of the baseline fluorescent signal, i.e., the normalised fluorescent signal of the first few PCR cycles. After reaching the threshold, the sample was considered positive.

Results:
Overall, the general clinical condition of the animals was good, no clinical signs for blood parasites or other diseases were observed. The size of the lymph nodes was normal and the mucus membranes had normal purple colour. Table 1 shows clinical parameters of the warthog (*Phacochoerus aethiopicus*), reedbuck (*Redunca redunca*) and water buck (*Kobus defassa*).

Table 1: Clinical values (mean ±SD) of warthog, reedbuck and water buck

Parameters	Warthog	Reedbuck	Waterbuck
Body temp.(ºC)	38.55±0.92	45.34±0.84	28.06±0.65
Pulse rate (Pulse/min.)	40.84±18.56	43.92±17.71	17.53±15.27
Respiratory rate (Breath/min)	41.46±10.65	29.46±31.90	26.76±17.82

Results are given as mean ±SD
Source: Constructed by authors

The mean haematological values for the warthog (*Phacochoerus aethiopicus*), reedbuck (*Redunca redunca*) and water buck (*Kobus defassa*) studied are shown in Table 2.

Table 2: Haematological values (mean +SD) of warthog, reedbuck and water buck

Parameters	Warthog	Reedbuck	Waterbuck
WBCs (10^3)	8.25± 1.23	2.45± 0.95	3.8± 0.72
RBCs (10^6)	5.03± 3.86	7.52± 1.22	11.27± 1.66
Hb (g/dl)	12.75±1.69	17.35± 1.23	16.78± 2.86

Results are given as mean ±SD
Source: Constructed by authors

Anaplasma spp. was detected in Giemsa-stained thin blood films of both warthogs and one of the two reedbuck blood samples. *Anaplasma spp*. appear in the infected erythrocyte as dense, homogeneously staining blue-purple inclusions 0.3-1.0µm in diameter (Figure 1). *Theileria spp.* was detected on the other reedbuck and waterbuck samples (Figure 2). Positive anti-*Toxoplasma* IgG antibodies were detected on the two warthog sera samples, but real-time PCR detected the specific DNA fragment (98bp) of *T. gondii* from whole blood in only one warthog (Figure 3).

Figure 1: *Anaplasma spp.* on reedbuck blood smear

Source: Produced by authors

Figure 2: *Theileria spp*. on reedbuck blood smear

Source: Produced by authors

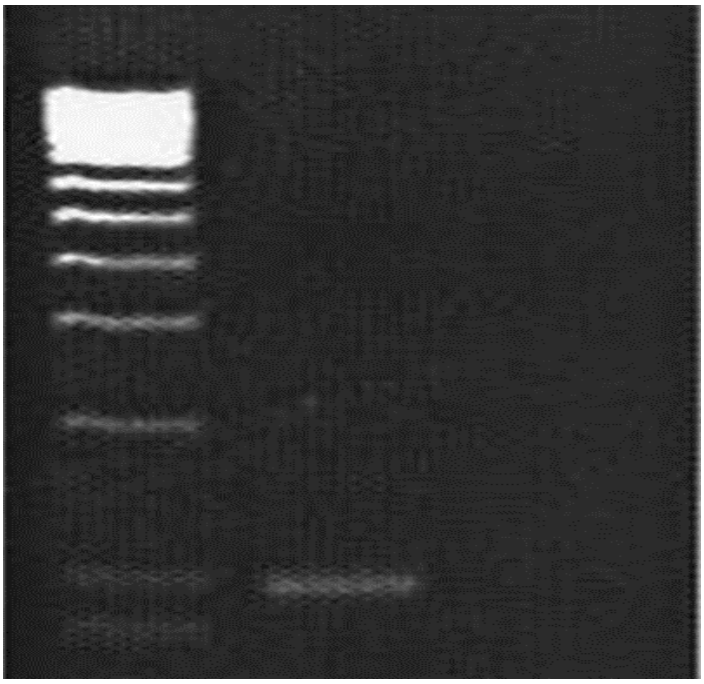

Figure 3: PCR product (98 bp) of B1 gene amplified from infected warthog with *T. gondii*

Lane 1: 50 bp marker, Lane 2: positive warthog sample, Lane 3: negative warthog sample
Source: Produced by authors

Discussion

The impact of livestock trespassing into protected areas can be felt in the Dinder National Park. Livestock compete with some wildlife species for food. Events of disease transmission from livestock to wildlife have taken place and the reverse is also possible. Most traditional grazing land around the park, which is also a habitat for migratory ungulates in the wet season, has been depleted.

The presence of wildlife in the National parks is one of the contributing factors for the transmission of diseases, as wildlife may act as reservoirs for the tick population and tick borne diseases (TBDs) (Kuttler, 1984). Wildlife-livestock interfaces are related to the opportunities for transmission of diseases common to both wildlife and domesticated animals. These disease problems are frequently bi-directional at the wildlife-livestock interface and therefore affect both domesticated animals and wildlife (Bengis *et al*., 2002). The present study detected the presence of *Theileria spp.* in reedbuck and waterbuck, *Anaplasma spp*. in warthogs and reedbuck, and *Toxoplasma gondii* in the warthogs' blood samples. The occurrence of *Anaplasma* antibodies in wildlife has been reported world-wide (Kuttler, 1984). The findings of this study confirm that wildlife carry *Anaplasma* organisms in Dinder National

Park and could serve as reservoirs of infection for domestic animals.
The animals examined in the present study did not show clinical signs for *theileriosis* or for other diseases; this is similar to observations made by Oosthuizen *et al*. (2009) in South Africa where *Theileria* piroplasms were detected in healthy free-ranging giraffes. No studies have been carried out that show the presence of *Theileria spp.* in wildlife in Dinder National Park, although tick species linked to *Theileria* transmission are widely distributed and have been collected from different wildlife species on game ranches, National Parks, and game management areas (Munang'andu *et al.,* 2012). Together with their transmitting vectors and wildlife reservoirs, *Theileria spp*. could be widely distributed in Dinder National Park.

The rising number of warthog may cause economic losses and represents a source of dissemination of different diseases. *Toxoplasma* infection is prevalent in a large number of animal species, affecting even zoo and free-ranging animals (Ullmann *et al.,* 2010; Mucker *et al.,* 2006). In this study, positive anti-*Toxoplasma* IgG antibodies were detected on the two warthog sera samples tested, while the real-time PCR detected the specific DNA fragment (98bp) of *T. gondii* from whole blood in only one warthog. Serological results show the passage of the parasite into the animal population, whereas molecular results detect its persistence into the population. PCR was used to detect *T. gondii because* it is very efficient in early diagnosis of toxoplasmosis (Tavassoli *et al*., 2009). Blood samples are the most available method required to perform PCR in the diagnosis of animal and human cases (Tavassoli *et al.*, 2009). Tissue cysts of *T. gondii* in meat of different game species are potential sources of human infection. The increased number of cases of parasitic infections is mainly due to the destruction of environmental conservation areas; this is driving wild animals out of their habitats and towards urban areas. High densities of free ranging cats are associated with farmsteads and urban areas (Van Wormer *et al*., 2013) and contribute *T. gondii* oocysts to the environment.

Body temperature and respiratory rates in the present study showed gradual elevation of their values. This may be attributed to the relative elevation in environmental temperature or may be due to chasing the animal for long distances. This result was in line with the findings of Ghobrial (1968) in captive Dorcas gazelles in Sudan, where higher levels of the rectal temperature measured in summer rather than winter were reported. A drop in eland's rectal temperature was associated with a slower respiratory rate (Taylor, 1969). The present study agrees with the findings of Taylor (1970) in six species of East African ungulates. The author reported physiological adaptation of gazelles through sweating and panting to reduce body temperature during the hot weather. Cloudsley-Thompson (1968) and Ghobrial (1967) also reported these findings in captive and free-ranging Dorcas gazelles. The heart rate of Dorcas gazelles was not affected by dehydration (Mubarak, 1986) or by immobilisation (Greth *et al*., 1993). In the present study, heart rate showed a progressive increase when animals were excited due to being chased. Hb and red blood corpuscles (RBCs) in the present study showed a slight increase in values. Fright or stress can cause significant changes in many haematological parameters (Diggs, 1966). Animals stressed for a short time showed an increase in circulatory erythrocytes; this might be due to splenic contraction (Hawkey *et al*., 1980; Jain, 1986). Compared to the immobilised capture used in this study, the effect of darting in the haematology of Grant and Thompson's gazelles was reported by Drevemo *et al.* (1974). The authors reported that there was a reduction in the values of RBCs, packed cell volume (PCV), and Hb, while there was an increase in mean corpuscular haemoglobin concentration (MCHC) values during anaesthesia.

Data from this study would help elucidate the role of *theileriosis* in the conservation of wildlife as well as improving our understanding of the epidemiology of toxoplasmosis, theileriosis and anaplasmosis in Dinder National Park. Therefore, we anticipate that the findings obtained from this study will stimulate the need for detailed investigations that will include molecular characterisation of different *Theileria spp*. and *Anaplasma spp*. found in wildlife, and further investigate the inter- and intra-species transmission of theileriosis in wild animals at Dinder National Park.

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Biography

Professor Aisha Elfaki Mohamed received her BSc in Veterinary Medicine from Zagazig University, Egypt (1984) and has worked as a Veterinary Officer since 1988 when she joined the Wildlife Research Center. She was appointed Head of Department of Wildlife Diseases, Breeding and Production, then Director of Wildlife Research, Wildlife Research Center. Her research covers wild animal health and production, wild bird diversity, and red-necked ostrich Struthio camelus camelus nutrition, diseases, behaviour, meat yield and quality. She is in expert groups (Lead Author) for the IPBES regional and sub-regional assessments of biodiversity and ecosystems, and the IPBES assessment of sustainable use of wild species. She is a Member of the National Core Planning Team for One Health Zoonotic Diseases Prioritization, CDC and WHO.

Manal Yousif Ishag Medani has a Bachelor of Veterinary Science from the Faculty of Veterinary Medicine, Zagazig University, Egypt (1987), an MVSc from the University of Khartoum, Sudan (1995), and a PhD from the University of Khartoum, Sudan (2003). She is an Associate Professor at CVRL, and specialises in parasitology with 29 years of experience in doing research on protozoa parasites (especially on cyst-forming coccidian).

Mohamed Siyab Eldin Hussain has a Bachelor of Veterinary Sciences from the Faculty of Veterinary Medicine, University of Khartoum (2000), an MVSc from the Faculty of Graduate Studies, University of Khartoum (2009), and a *Doctor scientiarum humanarum* (Dr. Sc. Hum.) from Eberhard Karls University of Tübingen, Germany (2013). He is a researcher with 13 years' experience of research on cancer, cardiology, nephrology and eryptosis, and has extensive teaching and technical advisory experience.