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## Prevalence and spectrum of helminths in free-ranging African buffaloes (*Syncerus caffer*) in wildlife protected areas, Tanzania

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### PEER REVIEW

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#### Comments

The authors demonstrated that GIT parasites are numerous in wildlife animals specifically buffalo. Cross transmission to domestic animals as well as humans could be possible with the prevailing interactions. Therefore this paper will form the basis for further studies.

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### ABSTRACT

**Objective:** To determine the prevalence and spectrum of helminths in free-ranging African buffaloes in Tanzania by a cross-sectional study.

**Methods:** Faecal samples ( $n=123$ ) from Arusha National Park and Ngorongoro Crater were examined for helminth eggs using sedimentation and floatation techniques during the period of March to June 2012.

**Results:** Coprological examination revealed that 34.1% ( $n=42$ ) of the buffaloes excreted nematodes and trematodes eggs and protozoan oocyst in their faces. The pattern of infection was either single or mixed. Single (52.4%) and concurrent infections with two, three, four and five parasites were recorded in 19.0%, 11.9%, 14.3% and 2.3% respectively of the cases. The nematode eggs encountered were those of *Trichostrongylus* sp. (20.3%), *Oesophagostomum* sp. (7.3%), *Strongyle* sp. (4.1%), *Bunostomum* sp. (4.1%), *Ostertegia* sp. (3.3%) and *Toxocara* sp. (2.4%). The trematode eggs encountered were those of *Fasciola* sp. (9.8%), *Paramphistomum* sp. (4.9%), *Gastrothylax* sp. (1.6%), *Ornithobilharzia* sp. (0.81%) and *Fischoederius* sp. (0.81%). The protozoan oocyst recorded was that of *Eimeria* sp. (8.1%). Geographical location of buffaloes had significant influence on the prevalence of infection with *Trichostrongylus* ( $P=0.046$ ) and *Fasciola* ( $P=0.001$ ), and the mean prevalences in Arusha National Park are significantly higher than those in Ngorongoro Crater. Age had significant influence on infection with *Fasciola* ( $P=0.036$ ), and juvenile recorded higher levels of infection than sub-adults. Health status, body condition score and sex-wise prevalence of helminths were not significant ( $P>0.05$ ).

**Conclusions:** This study indicates that helminths species are numerous and highly prevalent in the two protected areas and may be one of the contributing factors to lower buffalo productivity.

### KEYWORDS

Buffaloes, Endo-parasites, Protected areas, Risk, Wildlife, Tanzania

## 1. Introduction

It is a well-known fact that gastrointestinal parasitic infection in both livestock and wildlife is common and the

economic losses due to parasitic infection, causing mainly morbidity and sometimes animal mortality, are enormous[1]. The infection impact is greater in sub-Saharan Africa because the availability of a wide range of agro-ecological

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factors is suitable for diversified hosts and parasite species. Internal parasitism in buffaloes (*Syncerus caffer*) can result in loss of appetite, poor feed conversion efficiency and potentiation of other pathogens or even death<sup>[2–4]</sup>. The effects arising from direct losses may be visible but that of economic importance are indirect losses, which arise from sub-clinical infections such as decreased calf crop sizes, poor growth rates, reduced weight and visceral organ condemnation at slaughter<sup>[5–7]</sup>.

Buffaloes are even exposed to a higher risk of infection with snail borne helminths due to the animals' propensity to seek rivers, pools or swamps for wallowing<sup>[8]</sup>. In Tanzania, despite of numerous free-ranging buffalo population, knowledge and credible information on the prevalence, spectrum and intensity of helminths infections in wild range buffaloes are fragmentary, scanty and not available. Such knowledge of the spectra of parasites and their epidemiology is important for future research work and health education to consumers of bush meat.

The objective of this study was to conduct investigation aimed at shedding light on prevalence and the spectrum of gastro intestinal tract (GIT) parasites occurring in free-ranging African buffaloes in Tanzania.

## 2. Materials and methods

### 2.1. Study area

This study which constituted part of a broader survey on Foot and Mouth Disease was conducted in two wildlife protected areas namely Ngorongoro Crater (NC) in Ngorongoro Conservation Area (NCA) and Arusha National Park (ANP) located in northern Tanzania during the period of March to June 2012. The NCA (3°12'32" S, 35°27'46" E) which forms a part of the Serengeti ecosystem is a multiple land use area and was established in 1959. The NC is the world's largest intact, inactive caldera, occupying approximately 300 km<sup>2</sup> (4%) of the total NCA which has an area of about 8300km<sup>2</sup> lying at the western edge of the Great Rift Valley in northern Tanzania. The Crater floor (250 km<sup>2</sup>) is about 600 m below, composed predominantly of grassland interrupted by short bushes and scattered trees with small patches of swamps, Acacia and riverine forest. The NC is a World Heritage Site and Biosphere Reserve with high interaction of wildlife and livestock. Although a geographically distinct unit, it is part of the greater Serengeti Ecosystem (25000 km<sup>2</sup>) and regarded 'to some extent' as an ecologically distinct unit<sup>[9]</sup>. The NCA experiences two rainy seasons, long rains occurring between March–May and the short rains between November–January. Rainfall varies widely from 1000 mm in the highlands down to <600 mm in the semi-arid areas. Mean annual temperatures are between 13 °C to 28 °C and mean maximum temperature ranges from 10 °C to 32 °C. Vegetation cover is mainly

grassland interrupted by short bushes and scattered trees.

ANP, established in 1960 (3°15' S, 36°50' E) with elevation of 4566 m above sea level is one of the smallest park (322 km<sup>2</sup>) in Tanzania. The park is endowed with spectacular landscape and variety of wildlife. The main habitats include montane forest, grasslands, swampy areas, soda lakes, heath and upland desert on the upper slopes of Mt. Meru. The montane forest is evergreen and shows practically no seasonal variation. The mean annual rainfall is 900 mm (Momella lakes)–1300 mm (Ngurdoto caldera), sometimes as much as 2300 mm in the upper montane forest but an average of 200–250 mm up on Mt. Meru. At a height of 1500 m, the temperature during daytime is 20–28 °C and night time is 12–16 °C and is coolest in June–July. The park is isolated implying an absence of migration corridors for wildlife and a risk of loss of biodiversity. Illegal hunting, encroachment by farmers and livestock, and illegal logging of large trees for timber are the big challenge to the park authority.

### 2.2. Restraining procedure and data collection

A cross-sectional design in which data was collected at a single point and time was used. Animals were approached by a motor vehicle and immobilized according to protocols described by Kock *et al.*<sup>[11]</sup>. Immobilization drugs were prepared in dart syringes and delivered remotely by Dan inject<sup>®</sup> dart gun to selected candidate preferably on rump, neck or shoulder. Immobilization was undertaken by using Etorphine Hydrochloride (M99<sup>®</sup>, Norvatis Pty Ltd, South Africa) in combination with a tranquilizer Azaperone tartrate (Kyron Laboratories Pty Ltd, South Africa). Etorphine HCl was reversed by Diprenorphine Hydrochloride (M5050<sup>®</sup> Norvatis Pty Ltd, South Africa). All immobilized animals were examined for tick infestation and recording important bio-data such as age estimated by dentition and sex. For description purpose, buffaloes were grouped into 3 age categories: buffaloes under 5 years were classed as juvenile, those in the range of 5–10 years were classed as sub-adult and those above 10 years were classed as adults. Other information collected includes body condition score which was assessed visually and rated as poor or good and general health status at a time of sampling classified as healthy or unhealthy.

### 2.3. Sampling and parasitologic techniques

Faecal samples (fresh stool) were collected per-rectum using clean examination gloves, put into faecal pots, labelled and kept cool before transportation to the northern zone Veterinary Investigation Centre laboratory where they were immediately examined or stored at refrigerated temperature (4 °C) for a maximum of one day before processing. The sedimentation and floatation technique as described by Soulsby and Urquhart<sup>[12,13]</sup> was used to detect the presence

of stomach and intestinal eggs (trematodes and nematodes) in the samples. Identification of helminth eggs was done according to key described elsewhere<sup>[14,15]</sup>. The presence of coccidia oocysts was also recorded.

#### 2.4. Statistical analysis

Collected data were entered, stored and analyzed using Epi-Info version 6.04b (CDC, USA). Descriptive statistics were generated and presented as tables. For the epidemiological studies, the prevalence ( $P_i$ ) of buffaloes harbouring each parasite eggs/oocyst was calculated as  $P_i = D_i/N_i$ , where  $D_i$  is the number of buffaloes diagnosed as having a given parasite egg/oocyst at that point in time and  $N_i$ =number of buffaloes at risk (examined) at that point in time<sup>[10]</sup>. Associations between parasitism and categorical (host) factors were compared using *Chi*-square tests for independence. The level of significance was set at  $P < 0.05$ .

### 3. Results

#### 3.1. Descriptive statistics

Over all, 123 buffaloes were doted, faecal sample collected and screened. The average (Mean±SD) age of all investigated buffaloes was (6±3.95) years with range varying from 1 year to 20 years and the majorities (90%) of the animals were below 10 years of age. Of the 123 examined buffaloes, 68 (55.3%) and 55 (44.7%) were males and females respectively. All (100%) were found to be infested by various tick species and *Rhipicephalus appendiculatus* was the most predominant. The proportions of buffalo in each category of each variable investigated are detailed in Table 1.

**Table 1**

Proportion of each category investigated and the associated prevalence of parasite (all species) egg/oocyst infection.

Variable	Number examined	%	Prevalence (%)	$\chi^2$	<i>P</i> -value
Age category					
Juvenile	51	41.5	20(39.20)		
Sub adult	62	50.4	18(29.03)		
Adult	10	8.1	4(40)	0.58	0.7470
Sex					
Females	55	44.7	16(29.10)		
Males	68	55.3	26(38.20)	1.12	0.2890
Location					
ANP	25	20.3	24(96.00)		
NCA	98	79.7	18(18.40)	52.90	0.0021
Healthy status					
Healthy	120	97.5	41(34.10)		
Unhealthy	3	2.5	1(33.30)	0.54	0.4700
Body score					
Good	120	97.5	41(34.10)		
Poor	3	2.5	1(33.30)	0.54	0.4700

$\chi^2$  -*Chi*-square, *P*=Level of significance

#### 3.2. Prevalence of GIT parasite eggs/oocysts

A total of 123 buffaloes were sampled and 42 (34.1%) were positive for GIT nematode and trematodes eggs/oocyst, while 81 (65.9%) of the buffaloes sampled were negative for GIT parasites eggs/oocyst. The positive samples for trematode species were 15 (12.2%) whereas 37 (30%) samples were positives for nematode species and 10 (8.13%) samples were found positive for protozoan species. The proportion of the buffaloes harbouring nematode eggs of *Trichostrongylus* sp. was the highest (20.3%). Other nematode eggs encountered included *Oesophagostomum* sp. (7.3%), *Strongylus* sp. (4.1%), *Bunostomum* sp. (4.1%), *Ostertegia* sp. (3.3%) and *Toxocara* sp. (2.4%). Trematodes eggs were detected from *Fasciola* sp (9.8%), *Paramphistomum* sp (4.9%) and other fluke like species such as *Gastrothylax* sp. (1.6%), *Ornithobilharzia* sp. (0.81%) and *Fischoederius* sp. (0.81%). Protozoan oocysts (*Eimeria* sp.) were detected in 10 (8.1%) samples. Single (52.4%) and concurrent infections with two, three, four and five parasites were recorded in 19%, 11.9%, 14.3% and 2.3% respectively of the cases. Prevalence of GIT parasites are shown in table 2.

**Table 2**

Prevalence of different GIT parasites eggs/oocysts.

Parasite class	Parasite sp.	Number positive	Prevalence (%)
Nematodes	<i>Trichostrongylus</i>	25	20.30
	<i>Oesophagostomum</i>	9	7.30
	<i>Strongylus</i>	5	4.10
	<i>Bunostomum</i>	5	4.10
	<i>Ostertegia</i>	4	3.30
	<i>Toxocara</i>	3	2.40
Trematodes	<i>Fasciola</i>	12	9.80
	<i>Paramphistomum</i>	6	4.90
	<i>Gastrothylax</i>	2	1.60
	<i>Ornithobilharzia</i>	1	0.81
Protozoan	<i>Fischoederius</i>	1	0.81
	Coccidia oocysts	10	8.10

#### 3.3. Factors influencing the prevalence of GIT parasite eggs/oocysts infection

Significant factors influencing prevalence of GIT parasites infection are given in Table 1.

Prevalence varied significantly between location ( $P < 0.05$ ) of the buffaloes sampled, and the buffaloes sampled in ANP showing a higher risk [Relative Risk (RR)=5.23, 95% Confidence Interval (CI) 3.42–7.99] of GIT infection compared to those sampled in NCA (Table 1). Also, buffaloes from ANP recorded the highest prevalence of infection with *Trichostrongylus* sp. (40%) and *Fasciola* sp. (48%). Juvenile buffaloes recorded significantly higher levels of *Fasciola* sp. infection than sub adults ( $P=0.036$ ). There was no

significant difference in the prevalence of gastro intestinal tract parasites between males (38.20%) and females (29.17%) buffaloes ( $P>0.05$ ). With the exception of *Fasciola* sp., there were no significant differences in the prevalence of parasite infection among juvenile, sub–adults and adult buffaloes (39.2% vs. 29% vs. 40%;  $P>0.05$ ). Health status and body condition score at a time of sampling were not significantly associated with prevalence of parasite infection ( $P=0.747$ ).

#### 4. Discussion

The status of endoparasites in wild buffaloes in different protected areas in Tanzania is not clearly known. High prevalence of exposure to GIT parasite in the ANP (96% vs. 18.4%) was an unexpected observation. This suggests that the level of contamination, animal interaction and the presence of intermediate host between the two protected areas are broadly not the same and therefore more researches on factors contributing to such differences are necessary.

Based on egg structure, twelve different species of gastrointestinal tract worms and protozoan were identified in buffaloes. They were broadly classified as nematodes (6 species), trematodes (5 species) and protozoan (1 species) [14]. Factors such as level of host immunity, stage of parasite infection, other inherent park specific ecological factors (moisture, humidity, vegetation, soil, animal interaction) and the number of adult parasites in the GIT may be related to the broad and high level of GIT parasites eggs/oocyst detected in this study [2,16]. Consistent to the other studies, mixed infection (47.6%) involving more than one helminth genera was common in this survey and is in agreement with the results of other researchers [4,8].

*Trichostrongylus* sp. was the most prevalent in the two surveyed protected areas signifying their importance in the wildlife areas. However, the prevalence was significantly higher in ANP (40%) than in NCA (15.3%) ( $P<0.05$ ). *Trichostrongylus* species have a wide host range and high risk of cross infection between and among domestic and wild ruminants [17, 18]. *Trichostrongylus* infective larvae are resistant to harsh environmental conditions thus making this parasite species the most abundant helminth in semi–arid and arid regions [19]. The low prevalence of *Trichostrongylus* recorded in sub–adult to older animals are probably an indication of an increased resistance to infestation resulting in a decreased egg output by female worms [4,20].

*Strongylus* sp. was the third most abundant helminth

detected in this survey and the degree of infection is known to be higher in young animals than adults [19]. Its abundance is usually facilitated by its easy mode of transmission either by skin penetration or orally through ingestion of milk by young animals. Skin penetration is easy with young animals or those animals with moist skin living near water sources. *Fasciola* sp. were predominantly detected in ANP probably due to high environmental contamination with trematode eggs from encroaching livestock and presence of intermediate hosts.

Several researchers have reported the presence of different species of helminths in buffalo in Uganda, Zimbabwe and other parts of the world. Nakayima and Ocaido [21] identified 6 different species of helminths from buffalo in Queen Elizabeth National Park around Lake Edward in Uganda. Boomker *et al* [6] also reported *Agriostomum gorgonis*, *Cooperia fulleborni*, *Haemonchus bedfordi*, *Haemonchus contortus*, *Oesophagostomum radiatum*, *Parabronema skrjabini*, *Trichostrongylus axei*, *Trichostrongylus deflexus* and *Trichuris globulosa* in faecal samples of wild buffaloes in Kruger National Park, South Africa. However, the results of both the investigation deviated from the findings of this research work and the detection of internal parasites is concerned. The absence of some of the species from this study also attribute to the different geographical locations which influences the intermediate hosts of certain parasites. For example, liver flukes can only complete their life cycle in the presence of snails as the intermediate hosts which are found in dump areas. Other reasons are the differences in the fecundity of adult worms, pre–patent periods of these helminth species, age of animal, and their adaptation to the definitive host.

The sex of the hosts was not an important factor influencing the prevalence of helminth infection in this study despite the relatively higher prevalence recorded in male (38.2%) buffaloes than their female (29.1%) counterparts. This observation may suggest that the immune suppression associated with hormonal and or /immunological factors do not play a significant role in helminth transmission between sex [22].

Body conditions of the animal and the presence of ectoparasites (ticks) did not show any significant association with the prevalence of the parasites. The absence of association between body condition and prevalence disagrees with previous reports in other livestock species [23]. This could be explained by the fact that loss of animals' body condition in the study could probably attribute to other

factors, such as seasonal change of forageable feed stuff and the presence of other concurrent disease conditions common in wildlife areas.

In this study, no attempt was made to undertake detailed investigations such as helminth egg per gram of faeces, coprocultures and genetic analysis to determine the intensity of helminths burden and the species present in the study area. Furthermore, detailed investigation of other potential risk factors *i.e.* buffalo/other herbivores density and interaction and their association with helminths infection were not undertaken. Resource constraints affecting logistics and laboratory capacity were the main reason. However, a detailed epidemiological study to elucidate the seasonality, infestation pressure pattern of helminths, cross infection between animal spp. and the other predisposing factors such as moisture, humidity, *etc.* is therefore desirable.

The present study has revealed the presence of a range of helminths species, with ANP recording a higher prevalence than NCA. The presence of wide spectrum of GIT parasites (12 different spp.) with high prevalence is an indication that favorable environmental conditions for infection, survival and perpetuation exist in wildlife areas in Tanzania.

The mere presence of eggs and oocysts does not necessarily indicate clinical neither helminthosis nor coccidiosis. However, they do provide an indication of helminth species prevailing in free range buffaloes in Tanzania, an important step toward future research direction and programmes in GIT parasite in wild buffalo population.

Basing from the above findings it is apparent that, anthelmintic treatment is feasible needs for wild animals. However, the Wildlife Policy of Tanzania<sup>[24]</sup> does not give room for veterinary intervention to wildlife species that are not endangered or threatened. With such extensive protected areas in Tanzania of about 370000 km<sup>2</sup> (30%) of the land cover it is practically impossible to introduce routine veterinary intervention in wildlife. Both strategic and tactical methods of anthelmintics control like mineral licks treated with anthelmintics are made available to wild animals in game ranches in South Africa where the treated licks are strategically placed at specific locations like near watering points, salt leaks, breeding and grazing grounds<sup>[25]</sup>.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

The authors are grateful to Tanzania National Parks (TANAPA) for the permission to immobilize buffaloes in Arusha National Park (ANP). The Ngorongoro Conservation Area Authority (NCAA) Grant No: NCCA/12/02 is acknowledged for funding the disease surveillance in buffaloes in the Crater. United Kingdom–Biotechnology and Biological Sciences Research Council (BBSRC) Project Grant No: BB/H009302/1 in livestock and wildlife in northern Tanzania is thanked for the financial and logistic support during buffalo sampling in Arusha National Park. We would also like to thank the Directors, Directorate of Veterinary and Wildlife Services, for their kind permission to publish this work.

### Comments

#### Background

Gastrointestinal parasitic infection cause losses in wildlife mainly due to morbidity with severity in juvenile animals. The wide range of agro–ecological factors may perpetuates the infection not only in wildlife but also in domestic ruminants especially in interface areas.

#### Research frontiers

Due to the fact that reliable data on wildlife parasitic infection is inadequate, the current work indicates the importance of such information. Intervention based on animal behavior and controlling parasitic zoonoses due to wild meat would be feasible.

#### Related reports

Several other studies reported worm infestation in wildlife and its implications especially in young ones. These findings will be a road map for detailed epidemiology and the role as reservoirs where wildlife and livestock interaction is high.

#### Innovations and breakthroughs

Researchers revealed that GIT worms are prevalent in wildlife animals (buffalo) with variable effects. This is current and systematic report uncovering the problem in wildlife.

#### Applications

Further research is required to evaluate the implications

to other animals and zoonotic nature of some parasites. Also Deworming cattle in areas like NCA where commingling is high could be imperative.

#### Peer review

The authors demonstrated that GIT parasites are numerous in wildlife animals specifically buffalo. Cross transmission to domestic animals as well as humans could be possible with the prevailing interactions. Therefore this paper will form the basis for further studies.

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