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# *Cryptosporidium* infection in captive wild animals at Sanda Kyarimi Zoo in Maiduguri, Nigeria

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**Abstract**

Wild mammals are essential food sources for man and animal predators. *Cryptosporidium* species have a broad host range, including wildlife, which serve as crucial disease reservoirs for domestic animals and humans with a potential public health concern. The scarcity of information on the incidence of cryptosporidiosis among wild animals in North-eastern Nigeria necessitated the present study, which investigated the occurrence of *Cryptosporidium* oocysts among captive wild mammals in Maiduguri. Faecal samples collected from *Artiodactyla/Proboscidae* (n=9), Carnivores (n=7), Primates (n=9), Reptilia (n=4), and Rodentia (n=2) were examined using the Ziehl-Neelsen staining technique to detect *Cryptosporidium* oocyst. Of the 31 captive mammals examined, 17 (54.8%), 14 (45.2%), 9 (29.0%) and 22 (71.0%) were respectively classified as males, females, young and adults. A total of 12 (38.7%; 95% CI: 23.7, 56.2) out of the 31 examined samples were positive, with a higher prevalence of 57.1% (95% CI: 25.0, 84.2) observed among carnivores compared to the other animals. There was no statistical association ($p > 0.05$) between the occurrence of *Cryptosporidium* oocysts and sex as well as the age of the individual mammal species examined. This paper underscores the role of anthroponotic and zoonotic transmission at the human-wildlife interface in zoological gardens (Zoo) and parks worldwide.

*Keywords*: Captive Wild Animals; *Cryptosporidium* oocysts; North-eastern Nigeria; Rodents; Sanda Kyarimi park

## Introduction

Cryptosporidiosis (crypto) is an important gastrointestinal disease of many vertebrate species, including humans, domestic livestock, pets, and wildlife (Xiao, 2009; Ryan *et al*., 2014). It is caused by an Apicomplexan protozoan parasite of the genus *Cryptosporidium*, which has a worldwide distribution (Lv *et al*., 2009). The organism invades the gastrointestinal tract epithelium and produces mild-to-severe diarrhoea, depending on the immune status of the host organism (Leitch & He, 2011). Thus, the ingestion of oocyst via contaminated water or food (oral-faecal route) may result in (i) acute and self-limiting illness in immunocompetent host organisms and (ii) chronic life-threatening illness in young and/or immunocompromised hosts (Elwin *et al*., 2012; Kurniawan *et al*., 2013). In addition,

*Cryptosporidium* oocyst may be transmitted through inhalation, and respiratory disease has been reported in humans (Sponseller *et al*., 2014) and birds (Hamidinejat *et al*., 2014).

Recent phylogenic studies have identified nearly 29 species and 60 different genotypes of *Cryptosporidium* from a wide range of animal hosts and man (Li *et al*., 2015). Over 17 species of *Cryptosporidium* have been reported in humans (Ryan *et al*., 2014; Squire *et al*., 2017). However, *C. parvum* and *C. hominis* are currently considered the most common species causing disease in humans worldwide (Xiao, 2010; Ryan *et al.,* 2014). In the past, *C. parvum* and *C. hominis* were exclusively responsible for waterborne outbreaks, but, *C. cuniculus* from rabbits (*Oryctolagus cuniculus*) has been implicated in a single outbreak in the United Kingdom (Chalmers *et al*., 2009). *Cryptosporidium* species are generally considered serious threats to human health (Chen *et al*., 2002; Xiao *et al*., 2004), because many human species such as *C. hominis, C. parvum, C. cuniculus, C. ubiquitum, C. muris, C. andersoni, C. canis, C. felis, C. suis, C. fayeri, C. meleagridis, C. viatorum, C. scrofarum, C. erinacei, C. bovis, C. tyzzeri,* and *C. xiaoi* also infect domestic and wild animals worldwide (Salyer *et al*., 2012; Maurya *et al.,* 2013; Karim *et al*., 2014; Bodager *et al*., 2015; Wang *et al*., 2015; Zahedi *et al*., 2016b). Its extensive host range and geographic distribution, and zoonotic tendencies make *Cryptosporidium* species a great public health concern worldwide. *Cryptosporidium* oocyst is highly resistant to inactivation by commonly used disinfectants (Baldursson & Karanis, 2011; Burnet *et al*., 2014), and the oocysts from man, domestic animals, and wildlife may contaminate surface water (Ryan *et al*., 2014).

The Sanda Kyarimi Zoological Park was established in 1970 as a forest reserve, has transformed into a large wildlife sanctuary and a botanical park. It is situated within the Maiduguri metropolis. The Zoo measures about 169,969 $m^2$ (42 acres) and houses various wildlife species such as the crested porcupine, deer, bland cafes, crocodiles, hyenas, elephants, ostriches, pythons, and different snake species. The park also has a monkey village comprising several species of baboons and chimpanzees, attracting many visitors. The zoological park is drained by river Ngaddabul, which channels into lake Alau, the primary water source for Maiduguri and its surroundings. This is an essential focus for the potential zoonotic transmission of *Cryptosporidium* in Maiduguri (Mbaya *et al*., 2015).

Although there are several studies on *Cryptosporidium* infection in man and domestic animals, very few studies have documented the parasite in wild animals globally. For instance, only one study reported the occurrence of *Cryptosporidium* oocysts among wild animals in the arid zone of North-eastern Nigeria (Ibrahim *et al*., 2007). Therefore, this study was conducted to investigate the prevalence, host range, and zoonotic potentials of *Cryptosporidium* among captive wild animals in Sanda Kyarimi Zoological Park in Maiduguri, North-eastern Nigeria.

**Materials and Methods**

*Study area*

Maiduguri, the capital city of Borno State is in the North-eastern region of Nigeria within latitude $11^0 51^1$N and longitude $13^0 5^1$E with an altitude of 354m (1161ft). The estimated population of Borno state was 1,907,600 in 2007. The State shares international borders with the Republic of Chad - North-east, Niger - north, and Cameroon - east. It also borders the Nigerian states of Adamawa and Gombe to the south and Yobe to the west. The climate in the State is hot and semi-arid, with $35.2^0$C and $19.9^0$C average high and low temperatures, respectively. At the same time, the average precipitation and relative humidities are 552.1 mm and 30.2%, respectively. There is little rainfall during the year, with an average temperature of $25.8^0$C and annual mean precipitation of 613mm. Maiduguri metropolis is a Sahelian savannah with grasses, shrubs, and few trees.

*Sample collection*

Faecal samples were collected and examined from animals belonging to five different taxonomic groups of Wild animal comprising *Artiodactyla/Proboscidae* (n=9), carnivores (n=7), primates (n=9), reptiles (n=4) and rodents (n=2). A total of 31 fresh faecal samples were collected directly from the rectum of each animal, where possible, using disposable polythene gloves. Where it was impossible to obtain samples directly from the rectum, freshly voided faeces were collected using a wooden tongue depressor to carefully scoop the superficial layer without contacting the floor. The faeces were then packaged in individual nylon bags with labels according to the species, age and sex of each animal and immediately taken to the laboratory for processing and analysis. Samples not processed immediately were stored at a temperature of $4^0$C for one week until processed.

*Sample processing and microscopic examination*

A smear of each faecal sample ( 2.5cm x 4.0 cm) was prepared on a labelled clean, grease-free glass slide. The smears were air-dried and fixed by hovering 4 times over a Bunsen flame for 2 or 3 seconds. The modified Acid-fast stain (Ziehl-Neelsen stain) was used for its superior sensitivity and specificity (Rigo & Franco, 2002). After preparing the unconcentrated faecal smears, the slides were fixed with absolute methanol for 30 seconds, placed on a staining rack and flooded with cold Carbol Fuchsin. The slides were heated until steam could be seen rising from the surface, then rinsed with distilled water and allowed to dry. The stained smears were then decolourised with 3% acid alcohol for 30 seconds and rinsed immediately, followed by counter-staining with malachite green for 45 seconds. Finally, the slides were rinsed with distilled water and allowed to dry. A drop of immersion oil was spread over the smear and viewed directly using the oil immersion objective lens (x100). *Cryptosporidium* oocysts were identified as red coccoid bodies with thick walls measuring about 4-5 µm in diameter and containing thin-flat motile sporozoites on a pale green background using the WHO atlas of parasitic zoonoses.

*Statistical analysis*

The raw data were analysed using Microsoft Excel Spreadsheet Program Version 2016 to determine the proportion of animals having *Cryptosporidium* oocyst in their faeces according to species of the wild mammals tested. The Chi-square (Fisher's Exact) test were performed seperately for the wild animals and rodents in order to determine the associations between the occurrence of *Cryptosporidium* oocyst in the faeces and some independent variables (sex and age) using the Statistical Package for Social Sciences (SPSS) software version 22.0 [International Business Machines (IBM) Corp., Armonk, New York, United States of America]. The level of significance was set at $p \leq 0.05$ in all analyses.

**Results**

The results of the qualitative examination of faecal samples obtained from different species of captive wild animals and rodents in Sanda Kyarimi Park Maiduguri revealed *Cryptosporidium* oocysts in 12 out of the 31 examined animals with an overall prevalence of 38.7% [95% confidence interval (CI): 23.7-56.2]. Species-wise, *Cryptosporidium* oocysts were detected in 2 (22.2%; 95% CI: 6.3-54.7) Artiodactyla/Proboscidae, 4 (57.1%; 95% CI: 25.0-84.2) Carnivores, 4 (44.4%; 95% CI: 18.9-73.3) Primates, and 2 (50.0%; 95% CI: 15.0, 85.0) reptiles. However, this study detected no infection from rodent samples (Table 1).

Of the total 31 wild animals and rodents examined, 17 (54.8%) were males and 14 (45.2%) females. Sex-wise, *Cryptosporidium* oocysts were detected among 2 (33.3%) female Artiodactyla/Proboscidae, 2 (50.0%) male and 2 (66.7%) female Carnivores, 2 (50.0%) male and 2 (40.0%) female Primates, 1 (50.0%) male and 1 (50.0%) Reptiles (Table 2).

Nine (29.0%) out of the 31 examined animals were classified as young, while 22 (71.0%) were adults. Age-wise, *Cryptosporidium* oocysts were detected among 2 (25.0%) adults *Artiodactyla/Proboscidae*. There were also 2 (100.0%) young and 2 (40.0%) adult Carnivores infected with *Cryptosporidium* oocysts. Furthermore, oocysts were detected from 4 (80.0%) young Primates and 2 (66.7%) adult reptiles. The occurrence of *Cryptosporidium* oocyst was not significantly associated with age and sex of the individual wild animals and rodents examined ($p > 0.05$) (Table 3).

**Discussion**

This study has established the occurrence of cryptosporidiosis among the captive wild animals in Sanda Kyarimi Park, Maiduguri, North-eastern Nigeria in various species of wild animals, which is higher than the 22.7% reported by Ibrahim *et al*. (2007). This disparity may be attributed to the differences in sampling periods, sample sizes, species of animals

**Table 1:** Specie-wise prevalence of *Cryptosporidium* oocysts in captive wild mammals, reptiles and rodents in Sanda Kyarimi Park, Maiduguri, North-eastern Nigeria

| Order | No. Examined | No. Positive | Prevalence [% (95% CI)] |
|---|---|---|---|
| *Artiodactyla/Proboscidae* | 9 | 2 | 22.2 (6.3, 54.7) |
| Carnivores | 7 | 4 | 57.1 (25.0, 84.2) |
| Primates | 9 | 4 | 44.4 (18.9, 73.3) |
| Reptiles | 4 | 2 | 50.0 (15.0, 85.0) |
| Rodents | 2 | 0 | 0.00 |

**Table 2:** Sex-wise prevalence of *Cryptosporidium* oocysts in captive wild mammals, reptiles and rodents in Sanda Kyarimi Park, Maiduguri, North-eastern Nigeria

| Order | No. Examined | No. (%) Positive | |
|---|---|---|---|
| | | Males | Females |
| *Artiodactyla/Proboscidae* | 9 | 2/6 (33.3) | 0/3 (0) |
| Carnivores | 7 | 2/4 (50) | 2/3 (66.7) |
| Primates | 9 | 2/4 (50) | 2/5 (40) |
| Reptiles | 4 | 1/2 (50) | 1/2 (50) |
| Rodents | 2 | 0/1 (0) | 0/1 (0) |

Sex of the individual wild mammals and rodents was not significantly associated with the occurrence of *Cryptosporidium* oocyst ($p > 0.05$)

**Table 3:** Age-wise prevalence of *Cryptosporidium* oocysts in captive wild mammals, reptiles and rodents in Sanda Kyarimi Park, Maiduguri, North-eastern Nigeria

| Order | No. Examined | No. (%) Positive | |
|---|---|---|---|
| | | Young | Adults |
| *Artiodactyla/Proboscidae* | 9 | 0/1 (0) | 2/8 (25) |
| Carnivores | 7 | 2/2 (100) | 2/5 (40) |
| Primates | 9 | 4/5 (80) | 0/4 (0) |
| Reptiles | 4 | 0/1 (0) | 2/3 (66.7) |
| Rodents | 2 | 0/0 (0) | 0/2 (0) |

Age of the individual wild mammals and rodents was not significantly associated with the occurrence of *Cryptosporidium* oocyst ($p > 0.05$)

and other extrinsic factors in the study area. Moreover, Ludwig & Marques (2011) reported a lower overall prevalence of 19.6 % from a previous study on the occurrence of *Cryptosporidium* species oocysts in mammals at a zoo in the Rio Grande to do Sul, Southern Brazil. Furthermore, a significantly lower overall prevalence of 10.56% was reported by Gu *et al*. (2016) from a previous investigation on *Cryptosporidium* infections in wild animals in a zoo in Anhui province, China. The lower prevalence may be linked to the remote location of the Zoo in a mountain forest that limited the exposure of animals to foreign pathogens from domestic animal reservoirs. Another study on wild animals living in the Cascavel city park in Paraná, Southern Brazil, reported a relatively higher overall prevalence of 49.15% for *Cryptosporidium* specie (Snak *et al*., 2015). The high infection rate was attributed to the herbivorous feeding behaviour of most of the animals studied, which facilitates the ingestion of oocysts in the soil and vegetation.

The non-significant association between the sex and the occurrence of *Cryptosporidium* oocysts in the animals' faeces found in this study corroborates with previous reports (Ibrahim *et al*., 2007; Venu *et al*., 2013). While numerous studies reported the occurrence of *Cryptosporidium* oocysts in both diarrhoeic and non-diarrhoeic animals (Maikai *et al*., 2009; Ayinmode & Fagbemi, 2010), this study reports their occurrence in asymptomatic non-diarrhoeic animals. This finding is epidemiologically essential because of the potential role of captive animals as reservoirs of infection for human visitors and other livestock that graze in the area and drink from the river Ngadabul, which receives surface runoff from the drainage canals of Sanda Kyarimi Zoo. The high prevalence recorded in young Carnivores and Primates (wild mammals) agrees with previous reports on the prevalence of cryptosporidiosis in domestic animals (Xiao, 2010; Budu-Amoako *et al*., 2012; Zhang *et al*., 2013; Akinkuotu & Fagbemi, 2014; Yui *et al*., 2014). This may imply that young animals are more susceptible to infection by *Cryptosporidium* due to their naive immune status. In animals, including livestock and companion animals, the development of immunity to *Cryptosporidium* infection can be influenced by the species, breed, health status, and age. Generally, younger animals are more susceptible to *Cryptosporidium* infections, as their immune systems are still developing and may not be fully equipped to combat the infection effectively (Díaz *et al.,* 2021).

In Nigeria and other parts of the world, several studies have found zoonotic and anthroponotic species of *Cryptosporidium* in humans, and animals respectively, as well as waterborne outbreaks (Karanis *et al*., 2007; Chalmers *et al*., 2009; Akinbo *et al*., 2010; Robinson *et al*., 2011; Salyer *et al*., 2012;

Adamu *et al*., 2014; Li *et al*., 2014; Karim *et al*., 2014; Wells *et al*., 2015; Qi *et al*., 2015; Zahedi *et al*., 2016a). This ubiquity exhibited by the *Cryptosporidium* species poses a significant risk to public health. Interspecies transmission of *Cryptosporidium* results from direct exposure to water contaminated with sources of animal faeces and human sewage (Karanis *et al*., 2007). Thus, *Cryptosporidium* represents a significant public health hazard in humans, livestock, and wild animals worldwide (Fayer, 2004; Caccio, 2005). It has been recognised as a significant food-borne parasitic disease in places where vegetables are eaten raw or lightly cooked (Ozlem & Sener, 2005). Faeces, faecal-contaminated soil, or contaminated water are the primary sources of contamination of vegetables with zoonotic *Cryptosporidium* species (Damen *et al.,* 2007). In many developing countries like Nigeria, poor hygiene and low living conditions are responsible for the increased risk of food-borne infections (Bekele *et al*., 2017). Maikai *et al.* (2011) reported human cryptosporidiosis in Kaduna State. Other reports from the North-Central (Banwat *et al.,* 2003; Udeh *et al.,* 2008), eastern (Okafor & Okunji, 1994; Okafor & Okunji, 1996) and western (Reinthaler *et al.,* 1989) parts of Nigeria have also been reported. In Maiduguri, previous studies have shown that raw vegetables sold for human consumption were heavily contaminated with helminth eggs (Adamu *et al*., 2014). Thus, the importance of this investigation on the occurrence of *Cryptosporidium* in captive wild mammals (including rodents) and reptiles cannot be overemphasised due to the potential to contaminate the food system by faeces of the wild animals.

In conclusion, this study established the presence of *Cryptosporidiosis* among various wildlife species examined at the Sanda Kyarimi Zoological Garden Maiduguri. The occurrence of this zoonotic parasite among captive wild animals in the Zoo may serve as a potential reservoir of infection for human visitors that frequent the Zoo for recreational activities. Improved personal and environmental hygiene, regular testing and treatment of wild animals in the Zoo and public enlightenment will help to control the spread of the disease and potential outbreaks. Potential sources of infection such as domestic effluent discharge into public water sources, open defecation and improper refuse disposal should be regulated. Future studies, especially at a molecular level, are essential to better appreciate the transmission and epidemiology of *Cryptosporidium* infection at the human-wildlife-livestock interface.

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**Conflict of Interest**

The authors declare that there is no conflict of interest.

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