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Gastrointestinal parasites in *Papio anubis* (Olive baboons) of Yankari game reserve: Zoonotic concerns

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Abstract

The emergence and reemergence of zoonotic diseases from wildlife is a growing concern. In line with the One Health concept, which recognizes the interconnectedness of human, animal, and environmental health, it is essential to consider all these factors to achieve optimal health outcomes globally. This study investigated the prevalence of gastrointestinal parasites in *Papio anubis* (olive baboon) troops living in close proximity to humans. The Yankari Game Reserve, a wildlife park in Bauchi State, Nigeria, has diverse wildlife populations, including *P. anubis*. One hundred and fifty-one faecal samples from *P. anubis* were collected and examined using formalin ethyl acetate sedimentation and centrifugal flotation techniques, revealing the presence of ten (10) species of intestinal parasites, accounting for an overall parasite prevalence of 145 (96.03 %) in the observed population. The most prevalent parasite species among the *P. anubis* population was *Entamoeba histolytica*, with a prevalence of 106 (70.2%), followed by *Entamoeba coli* with a prevalence of 97 (64.2%). *Anclyostoma spp.* was the most common helminth species, with a prevalence of 73 (48.3%), followed by *Strongyloides stercoralis* with a prevalence of 58 (38.4%). The high prevalence of potentially pathogenic parasites, such as *Anclyostoma* spp. and *E. histolytica*, among the *P. anubis* population is of public health importance and underscores the need for further research to examine humans in close proximity with these animals for the presence of gastrointestinal parasites and compare for species similarities. The findings of this research may help to determine the likelihood of cross-species transmission which is critical to safeguarding both humans and animals as they share the same environment.

Keywords: Gastrointestinal parasites, Papio anubis, Yankari Game Reserve, Zoonotic

Introduction

Disease transmission is a major concern for wildlife conservation and of public health implications, especially within and outside national parks and reserves (Phillips *et al*., 2004). Globally, 60% of emerging diseases are zoonotic, with wildlife accounting for 75% of these occurrences (Jones *et al*., 2008; Pourrut *et al*., 2011; Africa *et al*., 2021). According to the Centers for Disease Control and

Prevention, zoonotic diseases are responsible for 2.5 billion cases of illness and 2.7 million deaths worldwide each year (WHO EMRO, 2022). The One Health approach recognizes the importance of public health, animal welfare, and pathogen transfer to and from wildlife populations (WHO, 2022). Parasites and infectious diseases significantly threaten wildlife populations and have become a crucial concern in conservation biology. For instance, the Ebola virus caused the death of 5,000 gorillas from 2002 to 2003, while yellow fever caused the death of tens of thousands of howler monkeys in Central and South America (The Leakey Foundation, 2020).

Primate species, such as *Papio anubis* (olive baboon), can harbour over 50 parasite species, and they are likely to shed countless infectious parasite stages into the environment in a single day (Müller-Graf *et al*., 1996; Pitchford & Visser, 1975; Nizeyi *et al*., 1999; Nunn *et al*., 2003; Nunn *et al*., 2006). Some primates are known to harbour parasites which include sexually transmitted viruses, insect-borne protozoa that cause malaria, and helminths that cause schistosomiasis and tapeworm infections (Nunn *et al*., 2006). Parasites can potentially contaminate food, water, or substrates when touched or handled, and some of these parasites present significant public health concerns. Given the physiological, behavioural, and genetic similarities between humans and primates, cross-species infections by the same parasites are likely, posing a significant threat to both humans and primates (Jones *et al*., 2008; Larbi *et al*., 2020).

As with some pandemics and epidemics, human interactions with wildlife and other aspects of nature are the primary factors that lead to such occurrences (UNESCO, 2021; Machalaba *et al*., 2021). At Yankari Game Reserve and other reserves and parks, tourists, workers, indigenous people, and animals interact daily (Larbi *et al*., 2020). *P. anubis* are known for breaking into tourist cars, rooms and interacting directly with human environment and belongings, and this may result in the shedding of parasites, eggs and cysts in the environment and items (Nunn *et al*., 2003; Nunn *et al*., 2006). Therefore, this study assessed the presence and prevalence of intestinal parasites in *P. anubis* in Yankari Game Reserve.

Materials and Methods

Study site

The study was conducted in the recreational area of Yankari Game Reserve known as the Wikki Camp (Figure 1), which provides various amenities, including studio apartments, sports facilities, food outlets and a natural warm spring (Wikki Warm Spring), which stands out as the largest spring in the reserve characterized by crystal clear water and a stable temperature of 31 degrees Celsius throughout the year (RefinedNG, 2020).

The Yankari Game Reserve is one of the most popular eco-tourism destinations in the West African sub-region (Olokesusi, 1990). It covers an area of approximately 2,244 square kilometres. The reserve is located on latitude 09°50' N and longitude 010°30' E at about 150-750m above sea level in Bauchi State,North-eastern Nigeria, in the southern portion of the Sudan Savanna Zone (Odunlami & Lake, 2003; Omondi *et al.,* 2006; Omotoriogun *et al.,* 2011). It is one of the few areas left in Nigeria where wildlife is protected in its natural habitat. It is an important refuge for more than 50 species of mammals and 350 species of birds (Ezealor, 2001; Odunlami & Lake, 2003; Omondi *et al.,* 2006; Atuman *et al.,* 2019).

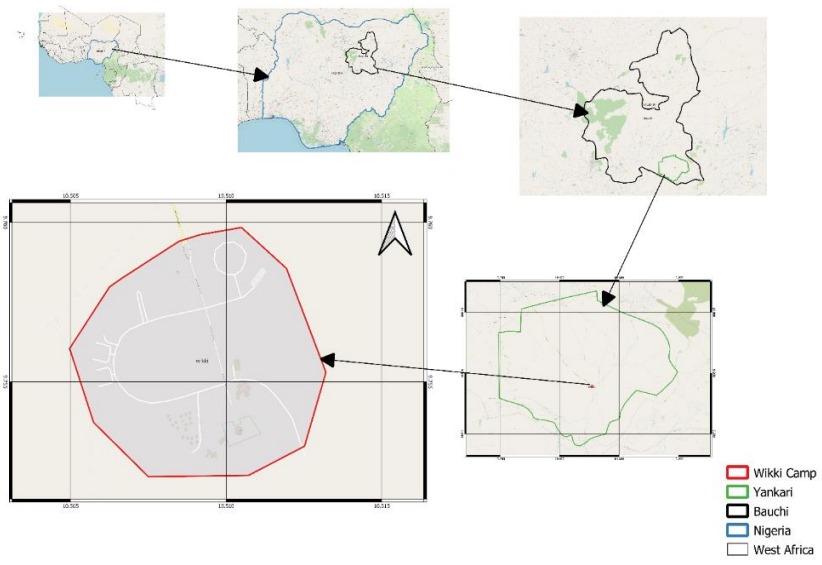

Figure 1: Map showing Wikki Camp in Yankari Game Reserve, Bauchi State, Nigeria

Study design

The study collected and analysed one hundred and fifty-one fresh faecal samples from the five different baboon troops that frequented the Wikki camp, to investigate the presence of gastrointestinal parasites. Each troop was trailed and as a random individual dropped its faeces, it was collected while still fresh. Each troop was made up of an average of thirty-five individuals. The collection of faecal samples spanned from November to December 2022. Subsequently, laboratory analyses were performed in January 2023 at the Molecular Ecology Laboratory, A.P. Leventis Ornithological Research Institute located in Amurum Forest Reserve, Jos, Plateau State.

Faecal sampling

P. anubis troops that made daily visits to the Wikki camp were trailed and care was taken to ensure that the presence of the researcher did not interfere with nor interrupt their daily activities. The collection of samples commenced at 0600 hours when the troops entered the reserve and ended at 1000 hours when they retreated into the forested area. Sampling resumed again at 1500 hours when the troops returned and ended at 1800 hours when they retreated for the night. Using sterile spatulas, fresh faecal samples were collected into sterile airtight sample bottles (Larbi *et al*., 2020). To avoid contamination from free-living nematodes, samples were taken from the middle of the faeces (Gillespie, 2006). The samples were preserved using 10% formalin (Larbi *et al*., 2021). The 10% formalin was dispensed onto the faecal samples immediately after collection using a sterile 10ml syringe. Formalin was used, due to its long shelf life and good preservation of helminth eggs, larvae, and protozoan cysts morphology (CDC, 2019). The sample bottle was vigorously shaken to increase the amount of contact between the sample and the preservative (Gillespie, 2006). The samples were labelled with details such as sample number, and date of sample collection and stored in air-tight zip lock bags. The sampling process was carried out for 25 days. Over the period of the 25 sampling days, a total of one hundred and fifty-one faecal samples were collected.

Faecal sample analyses

Concentration sedimentation and flotation methods were used in the laboratory to identify parasites in faecal samples rather than a direct wet smear because this procedure allows for the detection of parasitic elements (eggs, larvae, oocysts, and cysts) that may be omitted by direct wet smear (WHO, 2019). The sedimentation technique that was used was the formalin-ethyl acetate sedimentation concentration because it applies to both fresh and fixed faecal samples. This procedure is known to recover all protozoan cysts and oocysts, helminth eggs and larvae, and parasitic elements present in faecal samples. It is recommended as the simplest to perform and least prone to technical error, allowing recovery of the broadest range of parasitic elements (WHO, 2019). Centrifugal flotation was used to retrieve parasites that the formalin-ethyl acetate sedimentation could not retrieve. The centrifugal flotation method is a method that decreases the number of eggs that slowly rise to the surface of a flotation setup, unlike passive flotation. As a result, the number of false negative faecal examinations is reduced. Protozoan cysts, nematodes, cestodes, and some arthropod eggs are concentrated using this method (Veterinary Parasites, 1997). In general, centrifugal flotation techniques are thought to be more sensitive when it comes to recovering parasite ova. Centrifugation methods employ centripetal motion to aid in the suspension of helminth eggs and protozoan oocysts in a solution, as opposed to passive methods, which rely solely on specific gravity (relative density) of the flotation solution for parasite ascension. In this study, the flotation solution of choice was magnesium sulphate, which has a specific gravity of 1.2 and has the potential to recover common helminths and protozoan eggs/cysts (Burton & Lalande, 2021). All observed parasite species were identified using WHO (2019) bench aids for the diagnosis of intestinal parasites.

Formalin-ethyl acetate sedimentation concentration

This procedure was undertaken following protocols from Cheesbrough (2005).

One gram (1g) of preserved faeces is emulsified with 4ml of 10% formol water prepared by mixing 50ml formaldehyde stock solution with 450ml water. A glass applicator was used for the emulsification. Using a 350-micrometre sieve mesh, the emulsified solution of faeces and formol water was strained into a beaker. A funnel was used to transfer the strained solution into a 15ml centrifuge tube. To the already 4 ml of faecal solution in the centrifuge tube, another 4 ml of formol water was added. Four millilitres (4 ml) of stock ethyl acetate were added, and the centrifuge tube was covered with a stopper and shaken vigorously back and forth for 15 seconds to ensure that the entire solution was properly mixed. Due to the explosive nature of ethyl acetate, the stopper was then slightly loosened before being placed in the

centrifuge. For one minute, the solution was centrifuged at 3000 rpm. The solution was removed from the centrifuge and revealed four layers. The top layer is ethyl acetate, followed by the fat layer, formol water, and finally the sediment. The fatty layer is broken with a glass rod, and the top three layers are decanted to leave only the sediment. A pasteur's pipette is used to transfer a drop of sediment to a microscope slide. A drop of Lugol's iodine is added to the sediment drop on the glass slide, which is then covered with a coverslip. The prepared slide was then viewed under the microscope with the 10× objective lens, identification was done using 40×, and the presence of the various identified parasites was determined using WHO (2019) bench aids for the diagnosis of intestinal parasites.

Magnesium sulphate centrifugation flotation

This procedure was undertaken following protocols from (Clinical Diagnostic Parasitology, 2008; CDC, 2019).

A magnesium sulphate flotation solution was prepared by dissolving 400g of magnesium sulphate in 1000 ml of boiling distilled water using a heat block and magnetic stirrer. The solution was allowed to cool before it was transferred into wash bottles and allowed any remaining undissolved salt to sediment in the bottle before use. An estimate of 2g of faeces was emulsified in 10 ml of magnesium sulphate solution. Using a 350-micrometre sieve, this was strained into a beaker. A funnel was used to transfer the strained solution into a 15ml centrifuge tube. More magnesium sulphate solution was then added to the tube until it was about one inch from the top. The tube was covered and centrifuged at 1200 rpm for 5 minutes while the centrifuge was balanced. The tube was removed from the centrifuge carefully and placed in a tube rack without disturbing the solution. The tube's cover was gently removed, and drops of magnesium solution were added to fill the tube without disturbing the surface until a convex meniscus formed. A 10-minute timer was set and an 18mm-by-18mm coverslip was placed over the meniscus. After 10 minutes of flotation, the coverslip was carefully removed, leaving a drop of solution hanging from it, and placed on a microscope slide. The idea behind this method is that any parasites that are denser than the specific gravity of the magnesium sulphate solution (1.2g) will float to the top and attach to the coverslip. The entire coverslip was then examined with a 10× objective lens and a 40× objective lens for identification using the WHO (2019) bench aids for the diagnosis of intestinal parasites.

Statistical analyses

All data were inputted in Microsoft Excel 2019 and analyzed with RStudio 2022.12.0 build 353. In the analyses of the prevalence of parasites, the "epitools" which is a package in R studio, used in the analyses of epidemiological data was used to compute the percentage prevalence of each parasite species and their various 95% confidence intervals (Aragon *et al*., 2012)..

Results

Ninety-six percent (96.03%) (145) of the one hundred and fifty-one (151) *P. anubis* faecal samples tested positive for at least one intestinal parasite (Table 2) while four percent (3.97%) (6) tested negative for no parasites at all. The *P. anubis* were infected with a total of ten different species of gastrointestinal parasites. There were six helminths (Plate 1) and Four protozoan parasites (Plate II) (Table 1). The overall prevalence of intestinal parasites was 96% but some parasites were more prevalent than others. For example, *E. histolytica* recorded the highest prevalence of 70.20% while *Trichuris* spp. recorded the lowest prevalence of 0.66% (Table 2). The prevalence of the observed parasites in *P. anubis* was computed with their respective lower and upper limit confidence intervals at 95% confidence intervals. The 95% confidence intervals are the range of values above and below the prevalence estimate within which the true value in the sampled population is likely to lie (Table 2). Table 3 which shows findings pertaining to multiple parasitism revealed that

Table 1: Parasites identified in *P. anubis* faecal samples from Yankari game reserve, Bauchi, Nigeria

Helminths	Protozoans
Anclyostoma spp.	*Balantidium coli*
Ascaris lumbricoides	*Endolimax nana*
Hymenolopsis spp.	*Entamoeba coli*
Strongyloides stercoralis	*Iodamoeba buetschlii*
Strongylus spp.	
Trichuris spp.	

Table 2: Prevalence of helminth and protozoan parasites in *P. anubis* from Yankari game reserve, Bauchi, Nigeria (n=151)

	Parasites	Positives	Prevalence (%)	CI (95%)
Helminths	*Anclyostoma* spp.	73	48.34	40.5 - 56.3
	Ascaris lumbricoides	40	26.49	20.1 - 34.1
	Hymenolepis spp.	6	3.97	1.8 - 8.4
	Strongyloides stercoralis	58	38.41	31.0 - 46.4
	Strongyle-like eggs	31	20.53	14.9 - 27.7
	Trichuris spp.	1	0.66	0.03 - 3.70
Protozoans	*Entamoeba coli*	97	64.24	56.3 - 71.4
	Entamoeba histolytica	106	70.20	62.5 - 76.9
	Balantidium coli	43	28.48	22.5 - 36.8
	Iodamoeba buetschlii	94	62.25	54.3-69.6
Total		145	96.03	93.9 – 99.5

23.18% of the analyzed samples exhibited infestation by three distinct parasite species, which stands out as the highest proportion within the spectrum of multiple parasitism. Moreover, the results indicate that only 0.66% of the samples exhibited parasitic infestation by nine of the ten parasite species under observation in *P. anubis*. Overall, 88% of the samples were infested with 2 or more parasites.

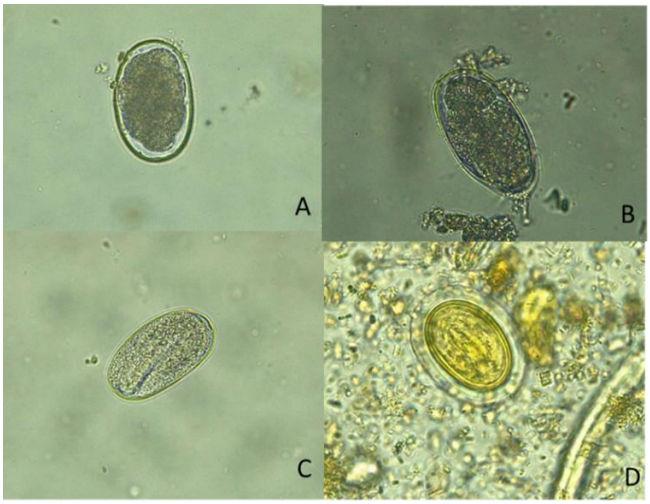

Plate I: Some helminth species observed in the *P. anubis* population of Yankari Game Reserve: (A) *Anclyostoma spp.* cyst, (B) *Strongyle-like egg*, (C) *Strongyloides stercoralis* egg with larvae, (D) *Ascaris lumbricoides* decorticated egg with larvae

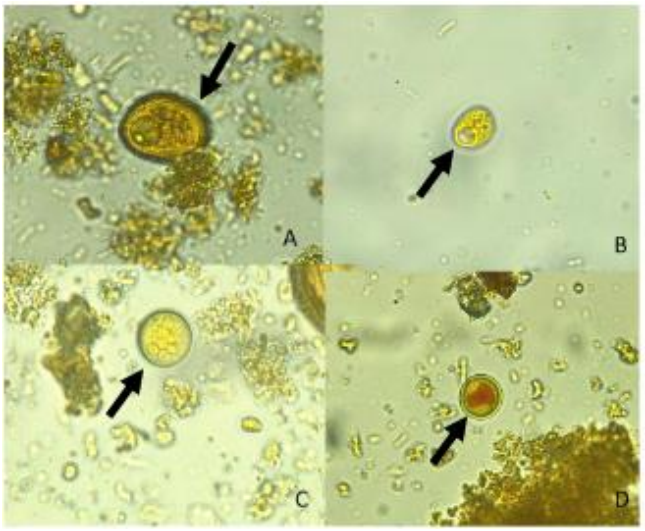

Plate II: Protozoan species observed in the P. anubis population of Yankari Game Reserve: (A) *Balantidium coli,* (B) *Iodamoeba buetschlii* cyst, (C) *Entamoeba coli* mature cyst with more than 4

Discussion

This research uncovered a helminth species (*Trichuris* spp) and also a protozoan species (*Balantidium coli*) in the Olive Baboon population that were not present in the previous studies by Mafuyai *et al*. (2013). However, Atuman *et al*. (2019) reported the presence of both Trichuris spp and Balantidium coli in other wildlife species in the same reserve, indicating possible cross-species transmission due to interactions. This study however identified 10 parasite species, observing 6 helminth species and 4 protozoan species. These protozoan species, *E. coli*, *E. histolytica*, and *I. buetschlii* still maintained a high prevalence in comparison to the previous study by Mafuyai *et al*. (2013). They recorded the highest prevalence with respect to both protozoans and helminths. Unlike the previous study by Mafuyai *et al*. (2013), which identified *A. lumbricoides* as the most prevalent helminth, *Anclyostoma* spp. was observed to be the most prevalent helminth parasite in the present population which conforms to studies in other countries where *Ancylostoma* spp was observed to be the most prevalent helminth species. (Pourrut *et al*., 2011; Larbi *et al*., 2020). The difference in this study and previously could be attributed to various factors such as transmission pathways and environmental conditions (Larbi *et al*., 2020). *Anclyostoma* spp., hookworm infection,

has a pathway of transmission which are; transmission of infective larvae through skin penetration or passively through ingestion of contaminated food or soil, which could explain the high prevalence observed in this study (Larbi *et al*., 2020). Similarly, *Anclyostoma* spp., *S. stercoralis* has the same two-way transmission (Greaves *et al*., 2013), which could explain the high prevalence of *Ancylostoma* spp. and *S. strongyloides* infections.

Table 3. Distribution of *P. anubis* faecal samples infected with 2 or more parasites (multiple parasitism) in Yankari Game Reserve (n=151)

Number of Parasites	Number of Samples	Percentage (%)
2	19	12.58
3	35	23.18
4	30	19.87
5	30	19.87
6	11	7.28
7	7	4.64
9	1	0.66
Total	133	88.08%

It is imperative to take into account the health implications of these gastrointestinal parasites parasiting *P. anubis* beyond their potential impact on human health. *Anclyostoma* spp. for example is associated with numerous health implications, including inflammation, ulceration, iron deficiency anaemia, protein malnutrition, dysentery, weight loss, and in serious cases, death in primates (Schmidt & Roberts, 1977). These intestinal parasites are of zoonotic and animal health implcations, because they are known to persist in *P. anubis* with significant morbidity (Larbi *et al*., 2020). The prevalence of the parasites among the *P. anubis* population in Yankari Game Reserve is a matter of significant concern. With an overall prevalence of 96% and 88% multiple parasitisms, the risk of morbidity is high. The results of this study emphasize the need for further investigation and immediate intervention to improve the health and welfare of this population. For instance, helminth infections in the population can be managed by administration of anthelminthic, such as fenbendazole, which has a broad-spectrum and has demonstrated effectiveness in the treatment of whipworms, nematodes, trematodes, and cestodes in both domestic and wild animals (Reichard *et al*., 2008).

Most helminthes identified in this study are highly pathogenic and of zoonotic importance. Humans infected with *A. lumbricoides* may experience symptoms such as diarrhoea, vomiting, and in rare cases, intestinal occlusion (Mafuyai *et al*., 2013). *E. histolytica*, the most prevalent intestinal protozoan found in *P. anubis* in this study, can cause amoebic dysentery and colitis in man. This infection is said to be responsible for an estimated 40,000-100,000 human fatalities globally each year (Ackers & Mirelman, 2006). The transmission of *E. histolytica* and *E. coli* from animals to humans is facilitated through faeco-oral route (Gruijter *et al*., 2005). *B. coli*, is known to affect both humans and non-primates in temperate and tropical climates (Ash & Orihel, 1997; Weyher *et al*., 2006).

In conclusion, this study determined the prevalence of gastrointestinal parasites in *P. anubis* troops in Yankari Game Reserve and the zoonotic potentials of the parasites. The high prevalence of potentially pathogenic parasites such as *Anclyostoma spp.* and *E. histolytica* within the troops emphasized the need for constant surveillance and monitoring to promote the health and productivity of *P. anubis* at the reserve. In addition, measures such as regular deworming, health checks, and improved sanitation of facilities could help reduce the risk of transmission between animals and humans as *P. anubis*-human interactions have also been identified as a potential route of transmission for these potential zoonotic infections. Regular workshops and training should be organized to educate workers and tourists to promote standard hygiene and best global practices, especially in a conservation facility such as the Yankari Game Reserve.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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