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Detection of cysts and coproantigen of *Giardia duodenalis* in domestic cats in Lokoja, Nigeria

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Abstract

Giardia duodenalis is a zoonotic upper intestinal tract parasite of humans, domestic and wild animals worldwide, causing diarrhoea in their hosts. *Giardia*sis is transmitted directly from person to person, animal to animal zoonotic or indirectly through water or food. Diarrhoea accounts for 16% in Nigeria, a country rated as having one of the worst prevalences of diarrhoea in Sub-Saharan Africa. The occurrence of *G. duodenalis* in cats poses a significant clinical and public health threat to people. This study investigated the prevalence of *Giardia duodenalis* in domestic (companion) cats in Lokoja, the capital of Kogi State, north-central, Nigeria. Faecal samples of 140 domestic cats were collected between November 2022 and October 2023 and analyzed for *Giardia cysts* using centrifugation-flotation and *Giardia* antigen using an immunochromatography assay. The prevalence of *Giardia* cysts detected was 1.4% while *Giardia* antigen was detected in 5.0% of the stool of the cats. The prevalence of *G. duodenalis* antigen in Tom cats was 5.8% and 3.8% in Queen cats while 18.2% of cats with diarrheic stool tested positive compared to the 0.9% of cats that were non-diarrheic. Based on age, the prevalence of *Giardia duodenalis* antigen was highest (8.0%) in cats that were less than 6 months old, while cats that were sampled in January had a higher prevalence of *G. duodenalis* (5.6%) than those sampled in December. The result suggests an association between faecal consistency and the prevalence of *Giardia duodenalis* in cats (P = 0.001). This study has confirmed the presence of *Giardia duodenalis* antigen in domestic cats in Lokoja. The occurrence of *Giardia duodenalis* in cats is of clinical and public health significance.

Keywords: Antigen, Cats, Cysts, *Giardia*, Immunochromatography, Lokoja

Introduction

Giardia duodenalis (syn. *G. lamblia* and *G. intestinalis*) is a single-celled flagellate protozoan parasite found in the small intestine of vertebrates including mammals. It has been included in the World Health Organization 'neglected disease initiative' (Medkour *et al*., 2020). It causes *Giardia*sis, a common intestinal infection. *Giardia duodenalis* is also referred to as *G. intestinalis* and *G. lamblia* (Rumsey & Waseem, 2024). Infection occurs in humans and a wide range of domestic and wild animals (Dixon, 2020). The severity of the disease depends on the age of the animal, nutritional status, management type and concomitant presence of other parasites such as *Cryptosporidium* and *Entamoeba* (Tawana *et al*., 2023). The prevalence of *Giardia* is high in calves under six months of age as compared to adults and may be responsible for diarrhoea (Mateusa *et al*., 2023). Only the species *G. duodenalis* has been recognized in cats causing subclinical infection, severe diarrhoea, weight loss, lethargy, poor

condition and mortality (Adam, 2021). Molecular data have identified seven assemblages (A to G) within *G. duodenalis*. Assemblages A and B have the widest host range that infects humans and a variety of domestic and wild animals while assemblages C, D, E, F and G appear to be host-specific for non-human species (Zajaczkowski *et al*., 2021). Others apart from assemblage G have however been isolated in humans (Zajaczkowski *et al.,* 2021).

Clinical signs are most likely to be seen in younger cats from multi-aged cat households (Veyna-Salazar *et al*., 2023). Transmission to humans is by faecal-oral route through ingestion of faeces or faecal-contaminated water, food or fomites. Of the two stages in the life cycle of the parasite, trophozoites are the active motile form. The trophozoites move towards the colon where they produce a cyst form and the cysts are extremely hardy and can survive for long periods in the water. The parasite has one to two weeks incubation period (Alharbi *et al*., 2020).

Diagnosis of *Giardia* infection traditionally is depended on microscopic identification of trophozoites or cysts in the faeces of affected animals. Centrifugation-Flotation is a reference method for the detection of *Giardia* cysts but however requires an experienced hand in microscopy because many artefacts mimic to varying degrees the morphology of *Giardia* cysts (Alharbi *et al*., 2020).

Several laboratory methods have been developed to detect the antigen in the faeces of infected cats. These methods include ELISA, immunofluorescence assay and molecular techniques which are highly sensitive, specific and more reproducible, but can be expensive and take time to be analyzed by a specialized laboratory. Commercial *Giardia* Antigen Test Kit (immunochromatography assay) are available for detection of *G. duodenalis* antigen in feline faeces. This test is a rapid enzyme immunoassay that can be conducted on fresh faeces or previously frozen faeces.

Many households in Lokoja keep cats as pets and rats' predators. They roam around residents and streets freely posing a great public health risk to humans and other animals. Lack of consideration for the health of these cats can jeopardize the health of humans. Currently, there is no published findings or data on the distribution of *Giardia duodenalis* in the domestic cat population in Lokoja. This study was therefore undertaken to determine the prevalence of *G. duodenalis* in faecal samples of domestic cats in Lokoja, North-central, Nigeria. The study also sought to establish if key variables of age of cats, season of sampling, faecal consistency and method of detection of *Giardia duodenalis* were risk factors of the occurrence of the parasite in Lokoja, North-Central, Nigeria. The results of this study can be important for cat owners and public health clinicians in the study area and beyond.

Materials and Methods

Ethics approval

Protocols of sampling of the animals were approved by the Faculty of Veterinary Medicine, University of Abuja Research Committee No 0202. Verbal informed consent from animal owners was given prior to the start of sample collection.

Questionnaire

An open-ended checklist was designed to collect information on individual cats. The questions included animals' biodata like age, sex, breed that will help us to identify risk factors of the disease. The studied cats were divided into two groups based on faecal consistency by visual assessment as diarrheic and non-diarrheic, age: three groups (group 1: <6 months, group 2: 6 months – 12 months and group 3: >12 months). Age was estimated by dental formulary and owner information. Classifications were also made based on sex by external genital examination and season. Questionnaires presented to the cat owners also helped to collaborate the data generated regarding sex and age of the cats in the study.

The questionnaire was administered through a semi-structured interview exercise in a Focus-group interview by our trained veterinary attendants. Furthermore, informed consent of the cat owners was verbally obtained from each participant before commencement and they were assured of confidentiality of all responses.

Study area

This study was carried out in Lokoja, capital of Kogi State, North central geopolitical region of Nigeria. It has a population of over 90,000 inhabitants (Brittanica, 2018). Lokoja lies about 7.8023° North of the equator and 6.7333° E east of the Meridian. It is about 165 km Southwest of Abuja. The town is situated in the tropical wet and dry savannah climate zone of Nigeria. The hot (dry) season lasts for 4 months, from January to April, with an average daily ambient temperature of above 33.889 °C. The rains last for 4.0 months, from June to October, with an average daily temperature below 30.56 °C (Brittanica, 2018). The occupation of the people of Lokoja includes farming, fishery and hunting. Lokoja is a trade centre for agricultural products situated at the confluence of the Niger and Benue rivers and close to

the Federal Capital Territory of Nigeria in (Brittanica, 2018).

Sample collection

Stools of all domestic cats were brought to the 6 major veterinary clinics (Veterinary hospital that has a qualified veterinarian) in the city for veterinary attention including vaccination, treatment against parasitic infections among others and satisfied the inclusion factors of the study were collected. Within this period, 144 cats were brought and enlisted but 4 of them died before the second and third samples could be collected. A total of 140 cats were therefore enlisted in the study.

A total of 8 trained veterinary assistants were recruited to help in the distribution of questionnaires and collection of faecal samples. Questionnaires were distributed to the cat owners to generate information such as the age and sex of the cats enlisted in the study.

The stool samples for this study were collected over 2 months from December 2023 and January 2024. Three separate two grams each of freshly voided faeces were collected at 48-hour intervals from the rectum of each of the 140 cats using disposable sterile hand gloves into appropriately labelled sterile plastic sampling vials. They were stored in an ice pack and taken for refrigeration in the Department of Veterinary Public Health and Preventive Medicine laboratory. The three separate faecal samples taken from each cat at an interval of 48 hours was to enhance the chances of detection of the parasite. This was made possible by arranging a follow-up visitation to the house of the cat owners after every 48 hours. These three separate samples from each of the 140 cats were pooled up and analysed for *G. duodenalis* oocysts. The samples were stored in an ice pack and transported to the Parasitology Unit of the Department of Public Health and Preventive Medicine Laboratory, University of Abuja, Nigeria where they were processed. Where the sample could not be immediately within three days, they were refrigerated under -20^{0}C. Excluded from this study were domestic cats not presented to the Veterinary clinic, those presented for surgery and all others suffering from trauma. Collection of the faeces was achieved by stimulating the anal region of the cat to defecate. Information regarding the sex and age of the domestic cats was obtained from their owners using a questionnaire. Faecal samples collected were analysed for *G. duodenalis* antigen by immunochromatography assay and cyst or trophozoite of *Giardia* in faeces by centrifugation-floatation and microscopic examination.

Laboratory analysis

Faecal centrifugation-flotation technique: This test was conducted according to the procedure outlined by Faust *et al.* (1939). Approximately 1 gram of the pooled stools (The 3 stools collected from each cattle at 48hrs intervals) of each of the 140 cats enlisted in the study was floated in 33% zinc sulphate solution (specific density 1.27). It was then filtered through gauze, and centrifuged in a 15 ml tube at 400g for 10 minutes. A drop of the float from the meniscus was examined microscopically at 400x magnification for the presence of *G. duodenalis* cyst (Dryden *et al.,* 2006). The floatation solution lyses the trophozoites and makes their detection impossible hence direct faecal smear was used for their demonstration.

Immunochromatography assay (Enzyme-Linked Immuno-sorbent Assay): In this study, we used RIDA®QUICK *Giardia*, a quick immunochromatographic test kit (Manufactured by R-Biopharm AG, An der neuen Bergstraße 17, D-64297 Darmstadt, Germany) for the detection of the antigen of *Giardia* in the stool of cats. The assay procedure was according to the method of Astiazarán-García *et al.* (2000). Frozen stool samples were thawed and brought to room temperature. The extraction buffer and the test cassettes were brought to room temperature of between 20 – 25 °C. The disposable test cassettes were only removed from their external packs shortly before they were used. The tests were carried out in a closed space to avoid direct sunlight.

Samples preparation: Extraction Buffer Diluent (1ml) was put in well-labeled test tubes. The liquid stool sample (100 µl) was pipetted with a disposable pipette (up to the second thickening) and suspended in a buffer placed in a tube beforehand. The solid stool sample (50 mg) was then suspended in the buffer. The sample was then homogenised by mixing on a vortex mixer. The homogeneous suspension was allowed to settle for at least 3 minutes until a clear supernatant is formed.

Testing the sample: Approximately 200 µl of the clear supernatant of the stool suspension was sucked up with micropipette and dropped into the round opening of the test cassette. Care was taken to ensure that the liquid flows through the membrane unimpeded as particles mistakenly pipetted may cause an obstruction and were therefore removed beforehand. The test result was read after 5 minutes.

Interpretation of results: For *Giardia* positive, visible red and blue bands are indicative of positivity to *Giardia* while negativity of a sample is when only the

blue band is visible. No visibility of any coloured band or combination of the colours is an indication that the sample is invalid. Changes in the colour of bands after 10 mins were considered invalid and were not used for any evaluation.

Statistical analysis

The data was summarized and presented as follows, cats were grouped by age, sex, season and diarrheic or non-diarrheic to determine whether these factors were associated with *G. duodenalis* infection. Statistical comparisons based on Chi-square and P-value to measure associations was carried out using IBM SPSS 25.0 statistical software for windows. Differences were considered significant when $P< 0.05$.

Results

Results showed 1.4% of the 140 faecal samples screened in the study were positive for *Giardia duodenalis* cysts using centrifugation-floatation-microscopy technique while coproantigen of *G. duodenalis* was detected in 5% of the same samples using immunochromatography technique. The difference between the methods was not statistically significant (X^2 = 2.87; df= 1; P-value = 0.90) (Table 1).

The prevalence of *G. duodenalis* was 5.8% (5/87) in males and 3.8% (2/53) in female cats in Lokoja. There was no significant difference between the prevalence of *Giardia duodenalis* in males and females (X^2 = 0.27; P-value=0.603) (Table 2).

The prevalence of *G. duodenalis* in cats aged < 6 months was 8% (4/50), those 6 - 12 months were 4.9% (2/41) while it was 2% (1/49) in cats over 12 months old. There was no significant difference in the prevalence between the age groups and the rate of occurrence of *G. duodenalis* (X^2 =1.31; P-value = 0.521) (Table 2).

The prevalence of *G. duodenalis* in diarrhoeal cats was 18.2% (6/33) compared to 0.94% (1/107) in the non-diarrheic group. There was a significant difference in the prevalence of *G. duodenalis* and the nature of stool in cats (X^2 = 15.79; P-value = 0.001) (Table 2).

The prevalence of *G. duodenalis* in cats during January was 5.6% (5/89) whereas it was 3.9% (2/51) in December. There was no significant difference in the occurrence of *Giardia duodenalis* and the monthly occurrence of *G. duodenalis* (P-value = 0.521) (Table 2).

Table 1: Prevalence of *Giardia duodenalis* in domestic cats in Lokoja based on different detection methods

Type of method	No of samples	No of positive samples (%)	Chi-square	P-value
Microscopy Examination	140	2 (1.4)	2.87	0.090
ELISA Rapid Test	140	7 (5.0)		

Note: No = Number; % = percentage; P-value = Probability value

Table 2: Factors associated with the Prevalence of *Giardia duodenalis* in domestic cats in Lokoja

Factors	Number of Samples	No positive samples (%)	Chi-square	p-value
Sex				
Male	87	5 (5.8)	0.255	0.613
Female	53	2 (3.8)		
Age				
<6 months	50	4 (8.0)	1.852	0.396
6 months-12 months	41	2 (4.9)		
>12months	49	1 (2.0)		
Nature of faeces				
Diarrheic	33	6 (18.2)	15.79	0.001
Non-diarrheic	107	1 (0.94)		
Monthly variation				
January	89	5 (5.6)	0.196	0.659
December	51	2 (3.9)		

Note: No = Number; P-value = Probability value

Discussion

Various techniques have been used to detect *Giardia.* The results obtained from these techniques vary significantly depending on their degree of sensitivity. In this study, centrifugation–flotation-microscopy technique and immunochromatography assay were used to detect *Giardia* cysts and antigens in the stools of the cats sampled. Higher detection of *Giardia* antigen using ELISA fast method (Immunochromatography) was recorded compared to detection of cysts of *G. duodenalis* using centrifugation–flotation-microscopy. Although the difference in the detection rate between the two protocols was not different, the result suggests a higher sensitivity of ELISA (Immunochromatography) over microscopy (centrifugation-floatation) in concurrence with the report of Uchôa *et al*. (2018) on higher sensitivity of ELISA over microscopy. The difference in the detection may partially be due to the intermittent excretion of *G. duodenalis* cysts and trophozoites in asymptomatic and symptomatic animals (Adam, 2021) making many *G. duodenalis* negative faecal results on microscopy not necessarily implying that the cat is not parasitized by the protozoan. Trophozoites rapidly disintegrate in faeces over time, thus reducing the likelihood of microscopic identification (Kasırga, 2019). Repeat examinations of three samples collected at 48-hour intervals from the same cat pooled together in this study, therefore, increasing the likelihood of positive results (Uchôa *et al*., 2018). The differences in prevalence using immunochromatography and microscopy protocols may therefore be attributable to the cyst loss as a result of disintegration which makes microscopy not sensitive enough to pick up the disintegrated ones (Kasırga, 2019) thereby making antigen assay by immunochromatography a better option of detection of *G. duodenalis.*

The prevalence of *Giardia duodenalis* in cats in Lokoja using immunochromatography assay in this study corroborates a report of the occurrence of *Giardia* in faecal samples of companion animals especially, cats and dogs, in Africa (Tawana *et al*., 2023). This result is also within the reported worldwide range of 0-52% prevalence of *G. duodenalis* in cats (Veyna-Salazar *et al*., 2023; Adam, 2021). Our literature search revealed no documentation on the prevalence of *Giardia duodenalis* in cats in Nigeria. Most documented works on the prevalence of *Giardia* in Nigeria were not in cats. Such reports included 58.2% in dogs in Ibadan, South Western, Nigeria (Adejinmi & Osayomi, 2010), 25.4% in pigs in Ogun State, Nigeria (Akinkuotu *et al*., 2019), and 10-19% in humans in Nigeria (Square & Ryan, 2017). Reports of a higher prevalence of 16% in cats in Sydney (Mundim *et al*., 2007) tallies with reports from Western Australia and USA that identified *G. duodenalis* to be the most common enteric parasite of domestic dogs and cats (Mateo *et al*., 2023). The differences in the prevalence among various localities may be due to the geographical ecosystem, detection method, age of the animal, whether symptomatic or not, where the animal was housed, level of hygiene, environmental sanitation and animal husbandry practices. Prevalence of *G. duodenalis* in male cats (5.8%) in this study is higher than in females (3.8%). There was, however, no difference in the prevalence of *G. duodenalis* and sex of the cats in the area. This result corroborates similar findings of Adam (2021) who observed that sex may not be a determinant in the infection but the territorial habit of the male domestic cat that makes it have a wider area of activity and exposure to the infection than females. Male cats generally have multiple partners (polygynandrous) in nature, giving them a wider range of activities that contribute to their higher probability of contact with parasites than their female counterparts (Szala & Shackelford, 2019). Results from the study showed that age may not be a risk factor in the epidemiology of *Giardia*sis in domestic cats in this study area. Differences in occurrence according to age grade were however apparent. In this survey, kittens < 6 months had a higher prevalence of *G. duodenalis* compared to the prevalence in older cats within the range of 6 months and 12 months. Adult cats > 12 months had the lowest prevalence. This finding tallies with that of Kurnosova *et al.* (2024) who observed that *G. duodenalis* cysts are more frequent in domestic cats under 12 months of age. Immaturity is considered a significant risk factor for *Giardia*sis in humans and dogs where immunity develops with age (Abeywardena *et al*., 2015). Kitten's behaviour of scratching surfaces and licking objects that may be contaminated may contribute to the higher prevalence of parasites in kittens. The development of humoral immunity especially with age leads to a decreased or outright cessation of the production of cysts as the cats advance in age (Abeywardena *et al.,* 2015). This may account for the declining prevalence rate of *G. duodenalis* in adult cats.

In this study, diarrhoeic cats had a higher prevalence of *G. duodenalis* compared to non-diarrheic cats. This finding agrees with the previous reports by Veyna-Salazar (2023) and Sursal *et al*. (2020) who observed cats with diarrheic stools to be significantly more

infected with *Giardia* than cats with non-diarrheic stools. Siwila (2017) observed that while *Giardia duodenalis* may be prevalent in dogs and cats, it rarely associates with the clinical disease as affected animals suffer minimal consequences of the disease though it may act as a source of zoonotic infection. Mosallanejad *et al*. (2010) had earlier associated kennel or cattery set-up where there is overcrowding with *Giardia*sis.

The area of this study, Lokoja, is situated in the tropical wet and dry savannah climate zone of Nigeria, where flooding usually occurs due to heavy rainfall. Monthly variation in the prevalence of *G. duodenalis* is thought to be a reflection of the effects of climatic changes on the parasite, the effect of rains on the environment, host physiology and photoperiod (Wang *et al*., 2023). In terms of monthly variation in the prevalence of *G. duodenalis,* higher prevalence of 5.6% was recorded in January compared to 3.9% in December. The heavy floods in the study area in the months preceding December when we embarked on sample collection may have, according to Noradilah *et al*. (2019), flushed *Giardia* from the contaminating stools of animals and humans in the environmental surface leading to a reduction in exposure rate of cats to *Giardia* cysts in the environment in December thus resulting in lower prevalence of the parasite in the month compared to the occurrence in January.

Available information on *Giardia*sis in Nigeria highlights the disease mostly in humans. Information on the disease in companion animals, cats in particular, in the study area, is hard to find. This work therefore, is a pioneer study on the subject in Lokoja, a city considered the gateway between northern and southern Nigeria. Data from this study forms an essential component for the evaluation of the zoonotic risk of the parasite in the area. Cats are generally companion animals for humans and children. The fact that children generally play outside in the soil makes them important targets of infection from *Giardia* cysts and trophozoites of infected cats which contaminate the environment through defecation in soil and waters. The presence of the parasite in this study therefore points to the zoonotic potential of household cats in the transmission of *G. duodenalis* to humans. It is also hoped that this result facilitates further studies in the molecular genetics of the *Giardia duodenalis* and its epidemiology in the study area using a larger sample size. This is because the sample size in this study limits our generalization of the status of the parasite in the area. Such further studies will be necessary to survey the overall epidemiological status of *Giardia* in stray cat populations, other livestock, and humans in Lokoja.

In conclusion, the result of this study confirms the presence of *G. duodenalis* cysts and circulating antigen in cats in Lokoja, North Central region, Nigeria. The result also suggested the nature of faeces as a likely risk factor in the transmission of *Giardia* in cats in the area.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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