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Prevalence and Diversity of Zoonotic Protozoa in Dogs in Lokoja, North Central, Nigeria

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ABSTRACT

The risk of zoonotic protozoan diseases has increased in recent times through the unregulated breeding of dogs in the neighbourhood of human dwellings. Dogs (341) brought into the seven major (Based on full employment of at least one veterinarian) veterinary clinics in Lokoja, North Central Nigeria (October 2021 to August, 2022) for medical evaluations were enlisted in this study to determine the prevalence and diversity of zoonotic protozoa in the dogs. Faecal and blood samples were screened using modified Ziehl – Neelsen and direct microscopy techniques for the presence of intestinal and blood protozoan oocysts. Out of the faecal and blood samples collected from each of the 341 dogs enlisted in the study, 207 (60.7%) were positive for protozoa. Faecal protozoa had a higher frequency of occurrence; 50.4% (172/341) compared to blood protozoan; 10.3% (35/341). *Eimeria histolytica* was the most frequently occurring protozoa; 14.4% (49/341), followed by *Giardia;* 12.0% (41/341) and the least was *Babesia;* 3.8% (13/341). There was however no significant difference in the prevalence of protozoa and the type of parasite (P = 0.702). Age-specific, prevalence showed that puppies under 1 year had a higher prevalence of protozoa; 84.1% (174/207) compared to the adults; 16.0% (33/207). The relationship was not significant (χ^2=3.816; P = 0.702). Local breed of dogs had the highest prevalence of protozoa; 68.2% (137/201), followed by exotic; 60.47% (26/43) and the least was the cross breed, 45.4% (44/97). There is a significant difference in the prevalence of protozoa in local and cross breeds of dogs (P = 0.001). The female dogs had a higher prevalence; 77.0% (117/152) compared to males; 45.5% (86/189). There was an association between prevalence of protozoa and sex of dogs (χ^2 = 16.77; P = 0.010).

Keywords: Diversity**;** Dogs; Microscopy; Prevalence; Protozoa; Zoonotic

INTRODUCTION

Dogs have been playing an important role in companionship, emotional support, and recreation to humans around the world since antiquity (Deng and Swanson, 2015). Dogs have evolved to occupy a unique position in the human world, unparalleled by any other successfully domesticated animal (Benz-Schwarzburg *et al*., 2020). The intelligence of these animals has been exploited by man, and this has made dogs useful to man for various activities, which include hunting, retrieving, herding, rescue operations, tracking and guidance (Benz-Schwarzburg *et al*., 2020). The unrestricted domestication of dogs close to human dwellings is rampant in developing countries, which, coupled with total lack of and/or insufficient veterinary attention and zoonotic awareness, accentuate the potentials of disease transmission (Okoye *et al*., 2011). Allergy, trauma, and more than 60 zoonotic transmissible infections have been reported to be consequences of canine parasitic infections due to the co-habitation (Traversa *et al*., 2014) and especially in children and immune-compromised individuals (Robertson *et al*., 2000). The risk of zoonotic infection from domesticated dogs is high in the developing countries due largely to the less restrictive obligations placed on owners of dogs (Edet *et al*., 2004). Transmission of these parasites to humans could be by direct contact with the dog and indirectly with dog excretions, open abrasions and wound, secretions, contaminated food and water (Lorenzini *et al*., 2007). With the increasing number of dogs from the 47,265-dog population in Lokoja in 2016 (Okeme, *et al*., 2016), there is an increased contact between the dogs and people thereby exposing humans to zoonotic infections. This study was therefore designed to determine the prevalence of protozoa (intestinal and blood protozoa) of zoonotic importance, their diversity, and the associated drivers of their infections in dogs in Lokoja, Kogi state, North central, Nigeria.

MATERIALS AND METHODS

Study Area

Lokoja is located at between latitudes 7^{0}45'N and longitude 6^{0}45'E and sits 55m above sea level (see Fig. 1) with a projected population of 246,101 inhabitants in 2014 (Ukoje *et al*, 2016) and total land area of 29,833 km2 (Adefisan and Egiku, 2018). Lokoja is characterized by wet and dry seasons, with yearly rainfall of between 1016 mm and 1524 mm and an average annual temperature of 27^{0}C (Alabi, 2009). It is located at the confluence of Rivers Niger and Benue and the people of the city sustain themselves through farming, fishing and hunting (Alabi, 2009). A total of 47,265 dogs were estimated to be in Lokoja mainly for hunting, security, breeding and companionship (Okeme, 2016). While most of the dogs were left to stray around the community to fend for themselves, a few others were kept indoor in cages or leash by their owners.

Study Population

A cross-sectional study was carried out from October 2020 to August, 2021 on a 341 dogs population presented to six Veterinary Clinics in Lokoja, Kogi State, North Central Nigeria. One day to 3-year-old dogs presented to the clinics on Tuesdays and Wednesdays of every week were enlisted in this study. Sample size was purposively determined by the total number of dogs brought to the clinics for medical attention within the sampling time frame from October 2020 to August 2021 and relevant inclusion factors. All dogs brought to the clinic for routine medical check-up, medical treatment, vaccination and other procedures other than surgeries were enlisted in the study. A total of 341 dogs brought into the clinics fulfilled these criteria and were enlisted in the study. The dogs were discriminated as local, exotic and cross of the two breeds according to the records kept at the clinics.

Data Collection Tools

Questionnaires were administered to dog owners through the Veterinarians participating in this study to obtain data on individual animals regarding their age, (Broadly divided into two as those below one year (<1 year) and those of one year and above (>1 year) using their dentition, sex (Based on sexual organs) and breed (Based on record kept at the clinics). The dogs were from those examined by Veterinarians for variety of reasons.

Copro-parasitological Investigation

Single fresh faecal samples (4g each) collected from the dogs using protective disposable gloves into clean and dry universal bottles were taken on ice packs to the Parasitology Laboratory section of the Federal Medical centre Lokoja where it was immediately stored in the refrigerator under 4^{0}c until processing for intestinal protozoa (Oocysts). They were later subjected to protozoan cysts and/oocysts detection using the Ziehl-Neelson microscopy technique earlier used by Tahvildar-Biderouni and Salehi (2014). The procedure involved concentrating the faeces by formalin-ether sedimentation method. Smears from the sediment (20 µL) were made and stained by the modified Ziehl-Neelson technique. This involves air-drying and fixing the smears with methanol for 2–3 minutes. The smear was stained with unheated carbol-fuchsin for 15 minutes. They were then washed off with water, decolorized with 1% acid alcohol for 10–15 seconds and again washed off with water after which they were counter stained with 0.5% malachite green for 30 seconds. The smears were washed off with water and the slide stood in a draining rack to dry. The oocysts were examined microscopically and identified using x40 power magnification according to Causape *et al*., (1996).

Haematological Screening

For blood protozoans, 1ml blood sample drawn from the cephalic vein of each of the 341 dogs by venepuncture using a 22g needle and transferred to tubes with anticoagulant (EDTA). They were then taken to the parasitology unit of the Federal Medical Centre, Lokoja, for screening of protozoan parasites. At the laboratory, each sample was divided into two. One half each used for blood smear for detection of Babesia and the other for *Leishmania* using microscopy. For *Babesia*, microscopic detection was according to the protocol of Dos Santos *et al*. (2021). Ten microliters of each of the blood samples was spread using a spreading slide on a flat surface of a glass slide to make a thin smear. The thin smear of blood was air dried, fixed in methanol and stained with Giemsa solution (Merck®, USA). They were then examined under light microscope with oil immersion at ×1000 magnification. Amastigotes of *Leishmania* detected were as described by Weina *et al*., (2004). They were ovoid or spherical measuring 1 – 5micrometre long by 1 – 2 micrometres wide possessing a large nucleus. The piroplasmids of *Babesia* which were round or oval and 1.5 – 3.0 micrometre in diameter were as described by Dos Santos *et al*., (2021). The piroplasm usually form a Maltese cross of four organisms.

Statistical Analysis

All data were analysed using EPINFO 3.5.3 software. The association between prevalence of protozoa infection and sex, age, and breed of dogs was determined by the chi-square test of association, calculated at 95% confidence interval. Statistically significant association is considered to exist between prevalence and tested variables when *P*-value is less than 0.05.

Ethical Approval

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 were given prior to start of the sample collection.

RESULTS

Of the faecal (Table 1) and blood (Table 2) samples from the enlisted 341 dogs analysed, 60.7% (207/341) were positive for zoonotic protozoan. Out of these protozoans, seven types of protozoans were detected in this survey. Broadly, 172 (50.4%) protozoans comprising *Entamoeba, Giardia, Cryptosporidium, Isospora* and *Toxoplasma* were faecal while 10.3% (35/341) comprising *Leishmania* and *Babesia* were blood protozoans of zoonotic importance.

Statistics of the protozoa detected revealed that *Entamoeba* had the dominating prevalence of 14.4% (49/341) (Table 1). This was followed by *Giardia* 12.0% (41/341), *Cryptosporidium* 11.1% (38/341), *Leishmania* 7.3% (22/341), *Isospora* 6.5% (25/341), *Toxoplasma* 5.6% (19/341) and *Babesia* 3.8% (13/341).

Of the dog population studied, 57.2% (195/341) were puppies of day 1 to 1year old (Tables 1 &2). The overall prevalence of protozoan parasites in this age group (day 1 to 1year old) was higher, 89.2% (174/195) compared to the 22.6% (33/146) in adult dogs. Prevalence of faecal protozoans in dogs <1 year old was 71.3% compared to 22.6% in dogs >1 year.

There was a diversified presence of protozoa across all groups of dogs screened. *Entamoeba* was more prevalent in the puppies, 21.0% (41/195) compared to the equal high prevalence of *Entamoeba* and *Giardia* 5.5% (8/146) obtained, each in adult dogs. While *Babesia* had the least prevalence of 3.8% (13/341) amongst the protozoa detected in this study (Table 1), it also had the least frequency of occurrence of 5.6% (11/195) in dogs <1 year old in the same way that *Leishmania* had the least prevalence of 0.7% (1/146) amongst other protozoa in adult dogs >1 year old (Table 2).

Table 2 showed that only two blood protozoans, *Leishmania* and *Babesia* were detected in the dogs studied. The frequency of occurrence of *Leishmania* 7.3% (22/341) is higher than *Babesia* 3.8% (13/341). The difference between the prevalence of blood protozoa and type of blood protozoa is not statistically significant (0.541). A greater proportion of blood protozoans were detected in younger dogs of <1year 16.4% compared to the 2.1% prevalence in dogs >1 year. Prevalence of *Leishmania* was 10.8% (21/195) in dogs <1 year old compared to the 0.7% (1/146) in those > 1 year while *Babesia* was 5.6% (11/195) prevalent in dogs < 1 year old compared to the 1.4% (2/146) in adult dogs > 1 year, 1.4% (2/146). There was no association between the prevalence of protozoa and the age of dogs in this study, (P = 0.702). While only one of the adult dogs had a single infection of *Leishmania*, majority of the puppies and adult dogs in this study had mixed and multiple infections.

Local breed of dogs (Table 3) had the highest prevalence of protozoa, 68.16% (137/201) among the three breeds of dogs screened. Exotic breeds came second, 60.47% (26/43) while the least prevalence was recorded in the cross breeds, 45.4% (44/97). An association was established between dog breed and prevalence of protozoa in the study (P = 0.001)

Sex-specific prevalence (Table 4) showed that female dogs in the study had higher prevalence, 77.0% (117/152) of protozoa compared to the males, 48.1% (91/189). There is a statistically significant association between sex and prevalence of protozoan infection (P = 0.010).

DISCUSSION

The overall prevalence of protozoa in dogs in this study was 60.7%. The high prevalence of protozoa in this study is like a study conducted in Ilorin, North Central, Nigeria in which a prevalence of 59.3% was established (Adedoja *et al*, 2015). Other similar high prevalence studies were 72.5% from Sokoto, Northwestern Nigeria (Mahmuda *et al*, 2012) and 58.2% from Ibadan, Southwestern, Nigeria (Adejinmi *et al*, 2010). Lower prevalence of 38% was however reported in Maiduguri, Northeastern, Nigeria (Mustapha *et al*, 2016). Prevalence of protozoa in dogs reported from other parts of the world were 90.6%, 25.6%, and 32.4% from Ethiopia, Egypt and Italy respectively (Mekibib *et al*., 2014; Ibrahim *et al*., 2016; Scaramozzino *et al*., 2018).

Table 1: Age-based distribution of faecal protozoa in dogs in Lokoja, North-central Nigeria

Faecal protozoa	**Prevalence (N=341)**	**Age Distribution (%)**		**Chi-square**	**P-value**
		<1 year (N=195)	>1 year (N=146)		
Entamoeba (49)	14.4%	41 (21.0)	8 (5.5)	3.816	0.702
Giardia (41)	12.0%	33 (16.9)	8 (5.5)		
Cryptosporidium (38)	11.1%	32 (16.4)	6 (4.1)		
Isoprora (25)	6.5%	17 (8.7)	8 (5.5)		
Toxoplasma (19)	5.6%	16 (8.2)	3 (2.1)		

Key: N = number

Table 2: Age-based distribution of Blood protozoa in dogs in Lokoja, North-central Nigeria

Blood protozoa	**Prevalence (N=341)**	**Age Distribution (%)**		**Chi-square**	**P-value**
		<1 year (N=195)	>1 year (N=146)		
Leishmania (22)	7.3%	21 (10.8)	1 (0.7)		
Babesia (13)	3.8%	11 (5.6)	2 (1.4)	1.225	0.541

Key: N = number

Table 3: Breed-based distribution of blood protozoans in dogs in Lokoja

Breed	**No. of Samples**	**No. positive n (%)**	**No. negative n (%)**	**Chi-Square**	**P-value**
Local	201	137/201 (68.2)	64/201 (31.8)	14.26	0.001
Exotic	43	26/43 (60.5)	17/43 (39.5)		
Cross	97	44/97 (45.4)	53/97 (54.6)		

Key: N = number

Table 4: - Sex based distribution of Protozoa in Dogs in Lokoja

Intestinal and blood Parasites (N)	Sex		Chi-square	P-value
	M (N=189) +ve (%)	F (N=152) +ve (%)		
***Entamoeba* (49)**	23 (12.2)	26 (17.1)	15.95	0.010
***Giardia* (41)**	12 (6.4)	29 (19.1)		
***Cryptosporidium* (38)**	12 (6.4)	25 (16.5)		
***Isospora* (25)**	12 (6.4)	13 (8.6)		
***Leishmania* (22)**	9 (4.8)	13 (8.6)		
***Toxoplasma* (19)**	9 (4.8)	5 (3.3)		
***Babesia* (13)**	7 (44.4)	6 (77.0)		

Key: N = number

The differences in prevalence could be due to the study location, hygiene practices, detection method used, type and age of dog. Protozoal diseases are insidious and thought to be transmissible by many asymptomatic dogs (Esch and Petersen, 2013). Majority of the protozoans detected were faecal against the relatively smaller occurring blood protozoans detected in the dogs. This may be due to the ease in transmission of the parasites. While the faecal protozoans are oral-faecal and can easily be picked from the environment, the blood protozoans require a vector especially arthropods for their development in their definitive host. *Entamoeba,* a faecal protozoon, had the highest and most diversified presence amongst the zoonotic protozoa detected. It was found in 14.4% of the dogs screened with substantial presence across sex and different classes of age of the dogs. The diversified presence of *Entamoeba* across parameters of measure in this survey corroborates the reports that the parasite is the most widespread protozoa in Africa, south America and India (Siwila *et al*, 2020). The result also agrees with the report that the commonest and important zoonotic parasitic protozoans especially the internal ones found in dogs presenting to veterinarians are *Entamoeba, Gardia and Cryptosporidium species* (Traversa, 2012). Earlier studies on *Cryptosporidium* in Lokoja by Adeiza and Nafarnda, (2011) had identified it as a pathogen with high potential to spread in the environment. In this study, *Gardia and Cryptosporidium* were similarly diversified in their presence across the factors of age and breed. The 11.14% prevalence rate of *Cryptosporidium* in this study agrees with the report that individual prevalence of *Cryptosporidium* in dogs was usually between 5.1% and 22.5%, with the highest level in puppies and declines with age while for *Gardia,* the individual prevalence was between 6.0% and 24%, with the highest level in dogs <6 months old (Hamnes *et al*., 2007). *Giardia duodenalis* and *Toxoplasma gondii* are reported to have high prevalence in companion animals (Ballweber *et al*., 2010; Jones *et al*., 2009). Giardiasis, caused by *Giardia duodenalis* with synonyms, *G. lamblia* and *G. intestinalis* is reported to be the most common pathogenic parasitic infection of humans has a high frequency of occurrence in this study only next to Entamoeba. Giardia is reported to be of bidirectional interspecies transmission from animals to humans with potential zoonotic risk and high rates of infection in animals and humans making it a major target of disease prevention (Feng and Xiao, 2011). In this study however, Toxoplasma has the least frequency of occurrence compared to the other detected zoonotic protozoans in dogs in the area. This may be because cats are regarded more as definitive hosts for Toxoplasma while dogs are mechanical carriers rarely shedding the parasites (Esch and Petersen, 2013). Isospora infections, sometimes called coccidiosis and caused by *Isospora canis* and a zoonotic protozoan, are common in puppies and less in adult dogs around the world especially where they are kept in kennels (Saari *et al*., 2019). In the study area, 6.5% of the dog population are infected. Of the 15 disease-causing species of *Leishmania*, 13 are thought to be zoonotic while dogs are thought to be the main reservoir of L. infantum for its role in the parasite transition from sylvatic to domestic transmission cycles (Gramiccia, 2005; Petersen, 2009). Contact with animals has generally been reported to increase the risk of giardiasis in humans. Transmission of *Leishmania* is though considered anthroponotic in humans, dogs have been suggested as reservoirs (Dereure *et al*., 2003; Bhattarai *et al*., 2010). *Babesia*, a blood protozoan parasite, had the least detectable presence of 3.8% amongst the dogs screened. Nalubamba *et al* (2011) described canine babesiosis as a vector-borne disease attributable to an intra-erythrocytic protozoa resulting in fever, anaemia, jaundice, splenomegaly, haemoglobinuria and thrombocytopaenia). Out of the many species of *Babesia*, *B. canis* and *B. gibsoni* are said to be the common cause of this disease (Kjemtrup *et al*. 2000; Nalubamba *et al*., 2011). Appearance in the erythrocytes has historically been used in identifying infection in dogs. The two species routinely found were often differentiated into large and designated as *B. canis,* whereas the small ones were termed *B. gibsoni* (Boozer and Macintire, 2003). A result from this study corroborates other reports of low-level prevalence of Babesia in dogs (Nalubamba *et al*., 2011; Garcia-Quesada *et al*., 2021). The difference in occurrence of these blood protozoa in dogs in the studies may be due to variation in season of sampling, environmental sanitation and nutritional upkeep, type, age and breed of dogs sampled. Adult dogs in this study generally had the least prevalence (0.68%) of protozoa. This finding concurs with the reports that protozoan parasitism in dogs is more of a problem of puppies than adult dogs due probably to the increasing immunity in the adult dogs (Regidor-Cerrillo, 2020). Although, age was not associated (P = 0.702) with prevalence of protozoa in this study, puppies had an insignificantly higher prevalence of the parasites than the adults. This high infections in puppies corroborates other reports of similar high incidence (Adedoja *et al*., 2015; Sebaa *et al*, 2021). The high prevalence may not be unconnected to the low passive immunity the puppies received from the bitches, thereby making them immuno-incompetent to resist the multiplication of the protozoa (Innes *et al*., 2020).

Prevalence of parasites in this study was highest in local breed of dogs compared to the exotic and cross between exotic and local dogs. The differences in prevalence may be due to the traditional extensive management method of rearing dogs in the study area. In this husbandry method, dogs roam free in search of food. They feed on carcasses of dead animals and birds. They are also coprophagic and generally feed on cats and human excreta where they pick the oocysts and cysts of these protozoa. While foraging around for food they also wantonly defecate and contaminate the environment with their contaminated faeces thereby spreading the infections. The low upkeep of most of these dogs, many times having poor owners and lacking good veterinary care, usually results in high nutritional deficiencies and attendant low immunity that enhances the vulnerability of the local dogs to protozoan infections. Infections in the exotic breeds may sometimes arise from consumption of contaminated water, food and kennels.

The female dogs in this study had higher prevalence of protozoa of 34% compared to their male counterparts (26.7%). This variation is probably due to the physiological peculiarities of the female dogs. The female peculiarities of pregnancy and nursing usually constitute stress factors that reduce their immunity to infections (Raza *et al*., 2018). Female dogs also, often harbour cysts and oocysts, which mobilize during pregnancies and infect subsequent puppies even when re-infections do not occur (Traversa *et al*., 2014).

Conclusion

There prevalence of zoonotic protozoan infections in dogs in Lokoja, North-Central Nigeria was 60.7% with *Eimeria histolytica* as the most frequently occurring protozoa, 14.4% (49/341), followed by *Giardia,* 12.0% (41/341) and the least was *Babesia,* 3.8% (13/341). Puppies, female, and local breeds had higher prevalence of protozoa than the adult dogs while the local breed of dogs similarly had higher prevalence of protozoa than their adult, male and their exotic and cross breed counterparts. We recommend that control measures of these parasites to mitigate the transfer of their infections from dogs to humans in the study area should involve measures targeted at these populations in dogs. The measures should include that proper sanitation of the environment upon which the dogs defecate be undertaken while personal hygiene of the dog keepers and the exposed children be enhanced as key control measures to protect humans in the area who are also at the risk of infections due to these protozoa.

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Conflict of interest

The authors do not have any conflict of interest to declare.

Authors' Contribution

AMA and NAS conceptualized and designed the study. NBUA, ECO AND EAG collected samples, analyzed data and drafted the manuscript of this research. AMA AND NAS supervised the study, interpreted data, analyzed data, and reviewed the document. All authors have reviewed and approved the final manuscript.

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