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Original Research
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Cryptosporidium and associated risk factors in local breeds of pigs kept in Donga Local Government Area Taraba State Nigeria

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ABSTRACT

Cryptosporidium, an enteric protozoan parasite, poses economic losses in animal production and has zoonotic potential. This study aimed to determine the prevalence of *cryptosporidium* and associated risk factors in local pig breeds within Donga Local Government Area of Taraba State, Nigeria. A cross-sectional study was conducted, and a total of 384 faecal samples were collected from pigs in eight sampling sites. The samples were examined using the modified Ziehl-Neelsen staining technique.The results showed an overall prevalence of 27.9% (107/384) for *cryptosporidium* oocysts. The prevalence was significantly higher in pre-weaned piglets (64%) compared to post-weaned (26.9%) and adult pigs (24.14%). Female pigs had a significantly higher prevalence (49.21%) than males (17.4%), and diarrhoeic pigs had a significantly higher prevalence (55.2%) than non-diarrhoeic (22.9%). The prevalence was higher in the rainy season (42.4%) than in the dry season (10.3%).The detection of *Cryptosporidium spp*. oocysts in the faeces of pigs suggests that these animals may serve as potential reservoirs and shedders of this protozoan parasite, contaminating the environment and serving as a source of infection to other animals and humans. Adequate personal and environmental hygiene, along with prompt veterinary care, should be intensified.

Keywords: Faeces, Oocysts, Prevalence, Protozoan, Ziehl-Neelsen

INTRODUCTION

Cryptosporidiosis is a worldwide disease caused by enteric protozoan parasites that infect various animals and humans (Jang *et al*., 2023). *Cryptosporidium species* is the causative agent of cryptosporidiosis in a wide variety of vertebrate hosts (Leoni *et al*., 2000). The parasite is protected by an outer shell that allows it to survive outside the body for long periods and makes it very resistant to chlorine disinfectants (Kosek *et al*., 2001). As a result, the oocysts of *Cryptosporidium* can survive for several months and retain infectivity in a latent form outside the host, despite adverse environmental factors, including salinity and chemicals (Smith *et al*., 2007). There are 44 known species and more than 120 genotypes of *Cryptosporidium* that have been identified (Ryan *et al*., 2021). Thirteen different *Cryptosporidium* species/genotypes have been isolated in pigs, including: *Cryptosporidium suis, C. scrofarum, C. muris, C. parvum, C. tyzzeri, C. homonis, C. meleagridis, C. felis, C. andersoni, and C. struthious. Cryptosporidium scrofarum* and *C. suis* infections account for more than 90% of cryptosporidiosis in pigs (Feng *et al*., 2018). Experimental studies have shown that pigs are also susceptible to infections with *Cryptosporidium hominis* and *C. meleagridis*, which suggested that pigs could be a source of infection for humans and other animals, posing an invisible threat to human health (Sheoran *et al*., 2012). Cases of human infection with *C. scrofarum* and *C. suis* suggest that these 2 *Cryptosporidium* species may be zoonotic (Sannella *et al*., 2019). The infectious stages of the parasite (*Cryptosporidium* oocysts) are shaded in faeces of infected pigs, and it spreads through the faecal-oral route, frequently through contaminated water (Paul *et al*., 2014). Infected pigs excrete oocysts in their faeces, which are resistant to environmental and weather conditions, and upon ingestion by a susceptible host, the oocysts are activated, releasing four sporozoites which then invade the host epithelial cells in the small intestine (Love *et al*., 2017).Transmission in animals mainly occurs via ingestion of oocysts excreted by infected animals especially neonates in overcrowded or mixed housing facilities. In humans, transmission occurs through direct contact, especially among people exposed to animals, particularly farm workers (Mahdi and Ali, 2002). Pigs with unrestricted movement are potential sources of environmental contamination with oocysts (Atwil *et al*.,

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1997). The disease is one of the most important diseases causing diarrhea in neonates and immune-compromised animals leading to large economic losses both directly (death) and indirectly (growth retardation and reduced productivity) in animals (Klein *et al.,* 2008). *Cryptosporidium*-associated diarrhea is either mal-absorptive; in which the infection results in loss of electrolytes and blunting of villi leading to a decrease in nutrient and water absorption by the intestines or may be secretive; in which there is secretion of chloride and carbonate ions into the intestinal lumen and decreased absorption of sodium chloride which results in the production of an osmotic pressure that forces water into the intestinal lumen (Foster and Smith, 2009). The diarrhea observed in cryptosporidiosis may be aggravated by coexisting infection with other enteric pathogens such as Giardia, Eimeria, salmonella, rotavirus, and helminths (Ayana *et al.*, 2009). When *Cryptosporidium* was co-infected with other enteric pathogens, pigs exhibited significant diarrhea and had a high mortality rate (Enemark *et al*., 2003).*Cryptosporidium* oocysts have also been detected in the stools of asymptomatic animals which implies that healthy pigs can also serve as carriers of the infection to other animals and man (Bjorkman *et al.,* 2003). Therefore, prevention of *Cryptosporidium* transmission in healthy pigs should be considered. The severity of the disease is affected by the host's age, sex, nutritional status, immune status, parasite species/subtype, and season of sample collection (Shirley *et al.,* 2012). The diagnostic methods employed in studies on cryptosporidiosis include microscopic examination of acid-fast stained faecal smears (Ayinmode and Fagbemi, 2010), and the more sensitive immunoassays such as the enzyme-linked immunosorbent assay and immune-fluorescence assay (Giadinis *et al*., 2012), and the highly sensitive molecular techniques such as PCR which is used to differentiate and characterize the *Cryptosporidium* genotypes found in animals and humans (Zhang *et al.,* 2013).In Nigeria, few studies have been carried out on cryptosporidiosis in pigs (Kwaga *et al*., 1988; Yatswako *et al*., 2007; Maikai *et al*., 2009). However, there is no published records of the disease in pigs in Donga LGA, Taraba State as of today. Therefore, this study is aimed at determining the prevalence and risk factors associated with cryptosporidiosis in pigs as a potential source of *Cryptosporidium* to humans and animals. This will help reduce the economic losses associated with the disease and safeguard public health.

MATERIALS AND METHOD

STUDY AREA

This study was carried out in Donga Local Government Area (LGA) of Taraba State, Nigeria. Located between latitude 7°43'N and longitude 10°03'E, with a land mass of about 3,121 km^2^ and also serves as the home of the Donga River which flows through the LGA. The LGA comprises of 10 political wards including Akate, Suntai-Daji, Kadarko, Mararraba, Gayama, Kumbo and Nyita. The average temperature of Donga is around 32°C, with an average humidity of 17% (Manpower Retrieved, 2022). Taraba state has a tropical climate, marked with two distinct seasons; wet season which starts in March and ends in October and dry season which starts in November and ends in March or April (Taraba state Diary, 2018). Donga has a population of approximately 177,900 people (NPC, 2006). The occupation of most of the inhabitants are farming, hunting, fishing, livestock rearing and trading.

SAMPLE SIZE DETERMINATION AND ETHICAL CLEARANCE

The sample size was estimated using Thrusfield formula ($N= Z^2P (1-P)/d^2$) (Thrustfield, 2007). Using 50% as the expected prevalence (P), 95% confidence interval (CI) (Z =1.96) and 5% desired absolute precision (d). The required sample size obtained by this formula was 384. All procedures involving the research in animals were approved by the Ethical Committee for Animal Research of Federal University Wukari Taraba State (Ref No:CAR/FUW/EL/010).

STUDY DESIGN AND SAMPLING METHOD

The study was designed as a cross sectional study, conducted between the month of August, 2023 and January, 2024, using convenient sampling techniques.

SAMPLE COLLECTION

A total of 384 faecal samples were aseptically collected directly from the rectum of the pigs that have not been de-wormed for at least 3 months using disposable latex gloves. For pigs in which rectal sampling was not possible, such as neonates, freshly voided faeces were collected by the use of wooden tongue depressors which were used to scoop up the superficial layer of the faeces without contacting the floor. The faecal samples were dropped into individual universal sample bottles containing 1ml of 10% formaldehyde for the preservation of the oocysts. The sample bottles were properly labeled based on stool consistency, age, sex, and location. Records on the season when the sample was collected was also recorded. The sampled pigs were categorized into pre-weaned piglets (up to 3 months of age), post-weaned piglets (above 3 months but less than 1 year in age) and adults (1 year and above in age). Age was obtained through oral interview or aging using dentition. On the other hand, the female pigs among them were identified by the presence of prominent vulva at the perineal area and udder located at the inguinal area. The presence of the male organ (scrotal sac) located at the inguinal area was used in the identification of the males (boars). The samples were

transported on ice packs to the Microbiology and Parasitology Laboratory of College of Agriculture, Science and Technology Jalingo, Taraba State for Parasitological analysis.

SAMPLE PROCESSING

Fecal samples were concentrated using the Formol-ether concentration method as described by Cheesbrough, (2005). The concentrated faecal sediment was mixed and dropped onto a clean glass slide, allowed to air dry, and fixed in absolute methanol for 3 minutes. The fixed smear was then stained using the modified Ziehl-Neelsen staining technique.The staining process involves applying carbol fuchsin stain and gently heating until steam rises, allowing it to stand for 5-7 minutes. Subsequently, decolorization with acid-alcohol is performed until no more color runs off. This is followed by counterstaining with methylene blue for 1 minute. Finally, the specimen is rinsed with water and air-dried. The stained smear was examined microscopically using 100X magnification for the presence of *Cryptosporidium* oocysts. A positive sample was considered when at least one oocyst with the correct morphologic characters was observed. *Cryptosporidium* oocysts appear spherical, 4-6 µm in size, containing a residuum and sporozoites, usually within a clear halo, and stain bright red against a blue background (Soulsby,1982).

DATA ANALYSIS

Prevalence of *Cryptosporidium* spp. were expressed in simple percentages and presented on tables. Association between various risk factors such as stool consistency, age, sex, and season and the occurrence of *Cryptosporidium* infection were determined using Chi-square test with the use of Statistical Package for Social Sciences (SPSS) version 17. In all the analysis, 95% confidence interval was used and value of $p<0.05$ were considered significant throughout the study.

RESULTS

OVERALL PREVALENCE

Out of the total 384 faecal samples examined, 107 were found positive for *Cryptosporidium spp.* oocysts, resulting in an overall prevalence of 27.9%.

PREVALENCE BY LOCATION

The distribution of cryptosporidiosis per location is shown in Table I. Donga town had the highest prevalence (38.3%), followed by Akate (32.7%), while the least prevalence was observed in Suntai-Daji (16.2%).

PREVALENCE BY AGE

The distribution of *Cryptosporidium spp.* infection according to age groups is shown in Table II. The pre-weaned piglets had the highest prevalence of 64%, which was significantly higher ($P<0.005$) than the rates observed in post-weaned piglets (26.9%) and adult pigs (24.14%).

PREVALENCE BY SEX

The female pigs showed a significantly higher prevalence (49.21%) than the males (17.4%) (Table II).

PREVALENCE BY STOOL CONSISTENCY

A significant relationship was observed between prevalence of *Cryptosporidium* infection and faecal consistency. Diarrhoeic pigs had a significantly higher prevalence (55.2%) than non-diarrhoeic pigs (22.9%) (Table II).

PREVALENCE BY SEASON

The prevalence of cryptosporidiosis was higher in the rainy season (42.4%) than in the dry season (10.3%) (Table II).In summary, the results showed that prevalence of *Cryptosporidium* infection was significantly associated with location, age, sex, stool consistency, and season in the study area. The pre-weaned piglets, female pigs, diarrhoeic pigs, and samples collected during the rainy season had higher rates of infection.

Table I: Distribution of Cryptosporidiosis based on Location

Location	Number examined	Number Positive	% Prevalence
Akate	58	19	32.7
Donga town	115	44	38.3
Suntai-Daji	37	6	16.2
Kadarko	43	11	25.6
Kumbo	58	10	17.2
Mararraba	41	9	21.9
Gayama	32	8	25
Total	**384**	**107**	**27.9**

DISCUSSION

The prevalence of 27.9% obtained in this study is comparably lower than previous reports in north-western states in Nigeria, such as 13.9% in Kebbi State (Yatwswako *et al.*, 2007), and 13.6% in Kaduna State (Maikai *et al.*, 2019), but lower than the 46.5% reported in Ogun State, south-western Nigeria (Akinkuotu and Fagbemi, 2014). In countries outside Nigeria, a much higher prevalence of 77.0% was reported in Ghana (Larbi *et al*., 2022), and 32.6% in Japan (Yui *et al*., 2014). However, Qi *et al*. (2020) reported a lower prevalence of 17.9% in China when compared to this study. The disparity in prevalence rates of different studies in Nigeria and outside Nigeria affirms the statement of Chen (2023) who reported that *Cryptosporidium* infection in pigs can vary between countries and also in different regions of the same country. This disparity may be linked to the diagnostic techniques employed in the studies,

Table II: Prevalence of Cryptosporidiosis in Pigs Based on Risk factors

Risk Factors	Number examined	Number Positive (%)	χ^2	*P-value*
Age				
Pre-weaned	25	16 (64)	17.71	0.000143
Post-weaned	156	42(26.9)		
Total	384	107(27.9)		
Sex				
Male	258	45 (17.4)	10.0996	0.001483
Female	126	62 (49.21)		
Total	**384**	**107 (27.9)**		
Stool consistency				
Diarrhoiec	58	32 (55.2)	25.6734	0.00001
Non-diarrhoeic	328	75 (22.9)		
Total	384	107		
Season				
Dry	174	18 (10.3)	48.5866	0.00001
Rainy	210	89 (42.4)		
Total	384	107		

sampling period, and differences in the ecological and environmental characteristics of different countries, or regions within a country. It has been reported that ELISA is more sensitive than the acid-fast staining method in diagnosing cryptosporidiosis (Yatswako *et al.*, 2007). Taraba State like another northern state in Nigeria where this study was conducted is characterized by short rainfall periods, high temperatures and low humidity while south-western Nigeria has contrasting ecological and environmental features characterized by low temperatures, relatively high humidity, and rainfall that could favor the survival of *Cryptosporidium* oocysts in the environment. This could be the reason for the low aforementioned prevalence rates reported in northern Nigeria as compared to Ogun, a southwestern state. This observation supports the finding of Fayer (2004) that high relative humidity and rainfall aid the survival of oocysts in the environment while high temperature reduces the viability and infectivity of the oocysts. Among the places covered in this study, *Cryptosporidium* infection was more prevalent in pigs kept in Donga town and Akate as compared to other places, probably due to their proximity to River Donga. *Cryptosporidium* spp. oocysts are likely to easily spread through rivers or groundwater to surrounding farms due to heavy rain, which increases the opportunity for pigs to be exposed (Jang *et al.*,2023).

Prevalence based on age was significantly higher in pre-weaned piglets than the post-weaned and adult pigs. This agrees with the reports of Nguyen *et al*. (2012), and Budu-Amoaka *et al*. (2012). This may be due to the underdeveloped immune system of the piglets which makes them susceptible to the infection. These pre-weaned piglets could be a major source of environmental contamination with oocysts (Graczyk *et al*., (1999). In contrast, Petersen (2020) and Chen *et al.* (2023) reported a higher prevalence of post-weaned and attributed the reason to weaning stress and/or reduced immunity resulting from the loss of maternal immunity as asserted by Li *et al*. (2018). The low prevalence observed in the adult pigs

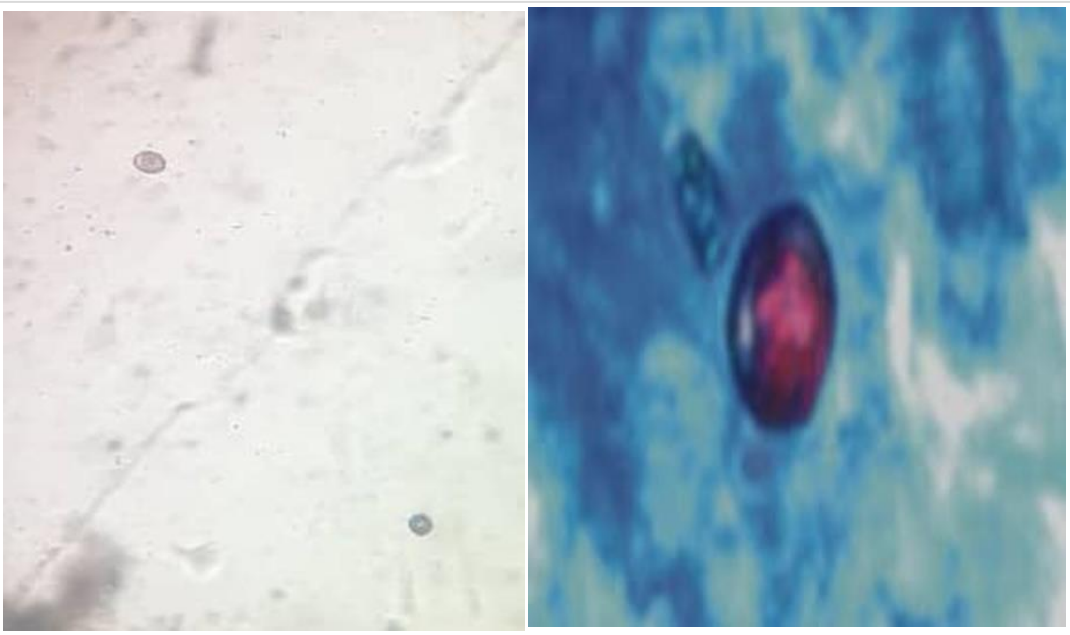

Figure I: The arrows 'A' indicates the presence of oocysts of *Cryptosporidium species* via faecal flotation method, *X40.* The arrow 'B' shows the oocyst of *Cryptosporidium species* via Modifield Ziehl-Neelson stain, *X100* against blue background

may be associated with a developed immune system that could combat the disease. Some of these adult pigs could also have developed immunity against the infection due to repeated exposure to the parasite. The confirmation of the disease in all age groups shows that cryptosporidium infection in pigs could occur over a wide age range (Olson *et al.,* (2004), and the significant variation in prevalence rates across the age groups signifies that the disease is associated with age factor (Featherstone *et al*.,(2010), and Maddox-Hyttel *et al*.,(2006).

Sex-related prevalence was significantly higher in female pigs than the males. This corroborates with the report of Akinkuotu and Fagbemi,(2014). The high prevalence observed in female pigs in this study could be due to environmental, management and strain-specific factor. It's possible that female and male pigs in this study were housed in different environments or have different levels of exposure to pathogens which could contribute to variation in disease prevalence. Differences in management practices between female and male pigs or between different farms can result in disease variation. It's also possible that certain strains of *cryptosporidium* have higher affinity for infecting female pigs or are more adept at evading the immune response in female compared to males. However, further study to understand the specific strain diversity and host-parasites interaction is crucial for elucidating why females may have a higher prevalence of *cryptosporidium spp*. Other authors such as Yang *et al*. (2017) and Maikai *et al.*(2019) reported contrary to our report, a significantly higher infection rate in male pigs. They pointed out that, male pigs are more likely than females to roam to other pens in search of females on heat to mount thereby at higher risk of contracting the disease and promoting the dissemination of the oocysts.

A significant relationship was found between the prevalence of *Cryptosporidium* infection and faecal consistency, with diarrhoeic piglets shedding the oocysts more frequently compared with normal or formed faecal consistency which agrees with the report of Caccio *et al.,(*2013). This may be possible because *Cryptosporidium* is an intestinal parasite that causes the loss of epithelial cells and microvillus of the intestine as well as a reduction in the absorptive surface area of the intestine resulting in diarrhea (Hamnes *et al*., 2006). The diarrhea observed may be associated with the pathogenesis of the parasite, and concurrent infection with other enteric pathogens such as salmonella, and *E.coli*. *Eimaria Spp,* Rotavirus and Giardia (Ayimonde and Fagbemi, 2010). This observation may also indicate that diarrhoeic animals in the study area may contribute to the spread of a high number of *Cryptosporidium* oocysts in the environment and could serve as a source of infection of water used on the farm (Harith *et al*.,2012). Although cryptosporidiosis is known to be related to diarrhea, pigs without diarrhea in this study also had *Cryptosporidium* oocysts detected in their feces. This may be due to the stage of the pathogen in the intestinal epithelium. The activities of the sporozoites and merozoites in the intestinal epithelia are responsible for the diarrhea seen in acute clinical cryptosporidiosis, thus a small number of oocysts may be shed in the feces at this stage. This means that non-diarrhoeic infected pigs can serve as reservoirs of the infections in other animals and humans. Subclinical chronic stage of the disease with *Cryptosporidium* oocyst has been speculated to exist in some instances without clinical signs of diarrhea (Maikai *et al*.,2019).

Seasonal-based prevalence was significantly higher in the rainy season than dry season in this study. This concord with the findings of Grackzyk *et al*.,(2000). Bern *et al*. (2000) also found that *Cryptosporidium* infections were most common in the rainy season. Heavy rain could cause the pigs to cluster together thereby increasing the chances of feed and water contamination with infected *Cryptosporidium spp.* Oocysts. During the rainy season, the floor is always wet, causing the survival of the *Cryptosporidium spp*. oocysts and consequently exposed the pigs to infection. Patz *et al*. (2000) asserted that factors such as rainfall influence the life cycle of *Cryptosporidium* and may influence the timing and intensity of disease outbreaks.

CONCLUSION AND RECOMMENDATION

This study shows that age, fecal consistencies, age and season influence the prevalence of Cryptosporidiosis in the study area. The detection of *Cryptosporidium spp*. oocysts in the faeces of the pigs suggests that these animals may be considered as a potential reservoir, and shedders of these protozoan parasites into the environment. The faeces from these infected animals could contaminate the environment, and serve as a source of infection to other animals and humans. Poor personal and environmental hygiene coupled with practice of using untreated pig's faeces as manure on vegetable farms could enhance the spread of this zoonotic disease in human population. Therefore, proper hygiene and prompt veterinary care of our animals should be practiced to minimize the spread of the disease and safeguard public health.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors

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