

Epidemiology of Cryptosporidiosis in Ruminant Species in Kebbi State, Nigeria

Danladi, Yusuf Kanya* and Ugbomoiko, Uade Samuel
Department of zoology University of Ilorin, Ilorin, Nigeria

Background: The zoonotic, parasitic diarrhoea disease *Cryptosporidiosis*, is a major constraint to livestock production throughout the tropics and beyond. However, the risk factors in ruminant species have not been sufficiently established in Northern Nigeria.

Methods/procedure: A cross sectional study on the prevalence of cryptosporidiosis and the factors influencing its distribution in ruminant species was carried out in two communities in Kebbi State, Nigeria. Faecal specimens were examined for *Cryptosporidium* by formal-ether concentration and modified Ziehl-Neelsen staining technique.

Results and findings: A total of 900 ruminant species were tested, 178 (19.8%) were infected with *Cryptosporidium*. Prevalence in cattle was 28.0% (98/350), Goats 17.1% (46/260) and Sheep 11.7% (34/290). Age related differences in prevalence were observed among goats ($P=0.029$), sheep ($P<0.001$) and cattle ($P<0.0001$). However such variations were not gender related in Goats ($P=0.658$) and sheep ($P=0.105$) but was related in cattle ($P=0.013$). Logistic regression analysis showed that cattle were about three times prone to infection than sheep and goats (Odds Ratio =2.510, P -value=0.004, 95% Confidence Interval =1.612- 3.909), diarrhoeal ruminants were significantly prone to infection than healthy animals.

Conclusion: Prevalence of cryptosporidiosis in ruminants is high in Kebbi State. Calves and diarrhoea condition are significant factors in the spread among ruminants and probably humans. Improved management system and hygienic practices must be embraced and promoted by owners.

Key words: *Cryptosporidiosis, Ruminants, Prevalence, risk factors, Diarrhea, Kebbi State*

I. Introduction:

Cryptosporidiosis is a significant disease in many agro-ecological zones being a serious threat to the livestock economy worldwide [1,2]. It is recognized as a major constraint to livestock production throughout the tropics and elsewhere [3]. The infection causes high morbidity and sometimes high mortality rates among domestic animals such as cattle, sheep, goat, pig and horses, resulting in serious economic losses [2,4,5,6,7]. Being zoonotic, infected animals pose health risk to humans especially in the immunocompromised individuals like people living with HIV/AIDS [8]. The parasite is considered as one of the major enteric pathogens associated with neonatal diarrhoea in cattle, sheep and goats [9,10]. Cattle are reported to be commonly infected with at least two distinct species namely *Cryptosporidium parvum* and *Cryptosporidium andersoni* [11]. Infection is primarily via the fecal-oral route and it takes less than 50 oocysts to infect a healthy animal [12]. Ingestion of environmental microscopic oocysts results in infection. Oocysts are discharged in the feces of infected animals and are significant in the dispersal and survival of the parasites [13]. The spread of oocysts can be rapid especially when animals are housed communally and overcrowded or from one animal to another via the udders when they are contaminated with infected feces [5]. Oocysts are resistant to harsh environmental factors and remain infective for months in cold water, damp or cool environments [11].

Clinical cryptosporidiosis may result in poor growth rate [14,15], severe diarrhoeal disease often characterized by anorexia and intermittent discharge [16,17]. The severity of clinical disease may be associated with the animals' immune and nutritional status [11]. Although young animals are most susceptible, the disease has also been found in older animals (eg cattle) over two years of age impairing rate of gain in feedlot cattle and milk production in dairy cattle [11]. Microscopic detection is based on finding the environmental and chemical resistant oocysts in fecal samples. Oocysts may be demonstrated using Ziehl-Nielsen fecal staining techniques of smears in which the oocysts appear as bright red granules [14].

In Nigeria, there are a number of reports on the prevalence of cryptosporidiosis, but its occurrence in small ruminant species has received little attention. Most studies have primarily focused on bovine cryptosporidiosis [18,19]. This paper reports on the epidemiology of Cryptosporidiosis and associated factors in the occurrence of the infection in Sheep, Goats and cattle in two communities in North-western Nigeria.

II. Materials and Methods

Study Area

Zuru is located in the south Eastern part of Kebbi State, between longitude 11° 24'09"N and latitude 5° 15' 07' E. It has an estimated population of about 165,547 thousand people living in the local government area. While, Aliero on the other hand is located in the extreme northwestern part, between latitude 12° 19'06"N and 4° 30' 10' E with a population of about 123,785 people. Population estimates based on the 2006 national census [20]. The two communities feature low socioeconomic status and poor environmental sanitation. Adequate water supply, sewage, and waste disposal systems are lacking. Garbage is burned or thrown away near houses and can be found deposited on several places. Zuru land supports the savannah kind of vegetation with pockets of woodland vegetation along the river basins. Grains, tubers, legumes, fruits are grown in the area. While Aliero is flat and slightly undulating with compact stony and brown soil, and has northern guinea savannah vegetation. The leading economic activity in both communities is mainly agriculture. The inhabitants therefore are mostly farmers, animal keepers, blacksmiths, traders and some are civil servants. The people of Aliero are predominantly of Hausa/ Fulani tribe, but Zuru people are Dakarkari by tribe.

Sample Collection and Handling

Visits were made to homes that gave their consent earlier before the sample collections commenced. Young and adult animals were sampled. For cattle, a calve of less than twelve (12) months was classified as young, while for Sheep and Goats, animals below six (6) months were considered to be young [21]. During the visits fresh rectal faecal samples were collected from each of the animals into a sterile, airtight, 10mL plastic tube. For animals in which rectal sampling was not possible, such as neonates, wooden tong depressors were used to scoop up the superficial layer of faeces without touching the floor. Collected faecal samples were labeled and transported in a cool box to the biology laboratory of Kebbi State University of science and Technology, Aliero (at least within 3 hours of collection) prior to dispatch in refrigerated containers for analysis.

Laboratory Analysis of Fecal Samples.

In the laboratory, stool samples were concentrated by formal-ether technique. Briefly using an applicator stick, about 1 g of stool sample was placed in a clean 15 ml conical centrifuge tube containing 7 ml formalin. The sample was suspended and mixed thoroughly with applicator stick. The resulting suspension was filtered through a sieve (cotton gauze) into a beaker and the filtrate was poured back into the same tube. The debris trapped on the sieve was discarded. To this mixture, 3 ml of diethyl ether was added and hand shaken; the content was centrifuged at 2000 rpm for 3 minutes. The supernatant was poured away, leaving only the fine sediment at the bottom of the tube [22]. This was then used to prepare slides for the detection of *Cryptosporidium* spp.

Ziehl-neelsen acid fast technique

One to two drops of fine sediment was smeared on the slide and air dried. This was fixed with absolute methanol for 2 minutes. The slide was flooded with carbol fuchsin for 15 minutes and rinsed thoroughly with water and decolorized with 1% acid alcohol for 2 minutes after which it was rinsed with water. It was then counter stained with malachite green for 1 minute and rinsed with water. This was finally air dried and examined under the microscope using the 10x objective. To achieve better view 40x objectives using oil immersion were used. Where present, *Cryptosporidium* oocysts appear round and stain red against a green to purple background. Samples were considered positive if at least one morphologically distinct *Cryptosporidium* spp. oocyst was observed [23].

Statistical analysis

Data collated at the end of the study were subjected to statistical analysis using the version 15 Statistical package for Social Sciences (SPSS Inc, Chicago, IL) on windows 10. Prevalence rates were calculated and presented in percentages. Chi square test was used to compare differences in prevalence for variables under consideration. A logistic regression analysis was carried out to assess the occurrence of *Cryptosporidium* infection among ruminant species. Values at $p < 0.05$ were considered significant.

Results

Out of the 900 ruminant animals examined, the overall prevalence of *Cryptosporidium* in all species was 19.8% (178/9000). Prevalence in cattle was 28.0% (98/350) while the proportion in Goats and Sheep were 17.7% (46/260) and 11.7% (34/290) respectively. Distribution of infection in cattle for the two study communities was significant ($P=0.004$), but infections in Sheep and Goats was comparable for the communities (Table 1).

Table1: Logistic regression analysis of species level occurrence of *Cryptosporidium*

Species	Number examined	Number Infected (%)	Odds Ratio (OR)	95%CI	P-value
Sheep	290	34(11.7)	1(Reference)		
Goats	260	46(17.7)	0.857	0.604–1.217	0.388
Cattle	350	98(28.0)	2.510	1.612–3.909	<0.0001

The occurrence of *Cryptosporidium* among the three species of study animals was compared using logistic regression analysis. A significant difference was observed among Cattle, Sheep and Goats. The likelihood of occurrence in Cattle was about three times more than its occurrence in Goats and Sheep (OR=2.510, 95% CI=1.612 – 3.909) whereas no significant difference was observed in Goats and Sheep (Table 2).

Table2: Prevalence of *Cryptosporidium* in ruminant species in Aliero and Zuru, Kebbi State

Animal species	Total Infection (%)	Aliero	Zuru	X ²	P-value
		Infected (%)	Infected (%)		
Sheep	34/290(11.7)	15/166(9.0)	19/124(15.3)	2.710	0.100
Goats	46/260(17.7)	20/114(17.5)	26/146(17.6)	0.003	0.956
Cattle	98/350(28.0)	44/200(22.0)	54/150(36.0)	8.333	0.004

Table 3 depicts prevalence with respect to infection in Sheep. Adult Sheep tended to be more infected than the young ones. However the distribution was not significant (p>0.05). Prevalence of the parasite was significantly higher in diarrhoeal stools than it was in the non-diarrhoeal (OR=4.318, 95% CI=3.205-5.817, P<0.0001). Although infection in male sheep was relatively higher than in females, the rates were statistically comparable (p>0.05).

Factor	Examined	Infected (%)	OR	95% CI	P-Value
Age					
Young	83	09(10.8)	0.514	0.376-0.704	0.0001
Adult	207	25(12.1)			
Diarrhoea Status					
With diarrhoea	87	22(25.3)	4.318	3.205-5.817	<0.0001
Without diarrhoea	203	12(5.9)			
Sex					
Male	152	14(9.2)	0.784	0.584-1.052	0.105
Female	138	20(14.5)			

Table 3: Relationship of age, sex and diarrhoea status with occurrence *Cryptosporidium* in Sheep at Aliero and Zuru (n= 290)

The distribution of oocysts in goats is summarized in table 4. While infection in young goats was significantly higher (27.1%) than in adults (14.9%), occurrence of oocysts was significantly common in diarrhoeal than in non-disrrhoeal goats. Diarrhoeal gaots were 3.432 prone to infection than the non-diarrhoeal (OR=3.432, 95%CI=1.744-6.755, P<0.0001). However, the distribution was not related to the sex of the animals.

Table 4: Relationship of age, sex and diarrhoea status with occurrence *Cryptosporidium* in Goats at Aliero and Zuru (n=260)

Parameter	Examined	Infected (%)	OR	95% CI	P-Value
Age					
Young	59	16(27.1)	0.447	0.217-0.920	0.029
Adult	201	30(14.9)			
Diarrheal Status					
With Diarrhoea	69	22(31.9)	3.432	1.744-6.755	<0.0001
Without Diarrhoea	191	24(12.0)			
Sex					
Male	130	24(18.5)	1.163	0.597-2.264	0.658
Female	130	22(16.9)			

Comparism of prevalence in Cattle is presented in table 5. Occurrence of oocysts was significant in distribution (<0.0001). Infection was about two times higher in calves (42.9%) than in adult animals (22.9%).

Diarrhoeal animals shedded significantly higher percentage of oocysts than non-diarrhoeal animals (53.6% versus 18.2%) and were more than six times likely to be infected than healthy animals (OR=6.153, 95%CI=3.691-11.553, P<0.0001). Also, prevalence in cattle was observed to be sex dependent (p = 0.001). Female animals discharged more oocysts (36.9%) than males (20.7%).

Table 5: Relationship of Site, Age, Diarrhoea Status and Sex with occurrence *Cryptosporidium* in Cattle (n=350)

Factor	Examined	Infected (%)	OR	95% CI	P-Value
Age					
Young	98	42(42.9)	0.246	0.138-0.439	<0.0001
Adult	252	56(22.2)			
Diarrheal Status					
With diarrhoea	97	52(53.6)	6.153	3.691-11.553	<0.0001
Without diarrhoea	253	46(18.2)			
Sex					
Male	193	40(20.7)	0.515	0.305-0.871	0.013
Female	157	58(36.9)			

III. Discussion

Various epidemiological factors account for different levels of parasitic infections in different settings. Cryptosporidiosis is prevalent the world over but with variable levels of infection in different host categories and climatic environments.

Prevalence rates reported in Nigeria among cattle include; 23.4%, [24], 30.6% [3], 37.5% [25], 33.0% [26], 28.0% [[27]. Others from other parts of the world include; 19.2% among calves in Zambia [28], 17.6% in central Ethiopia [29], 18.5% among cattle in Brazil [30], 24.0% among goat kids in Romania [31], 24.5% among lambs in Australia [32]. These reports are consistent with the 28.0%, 17.7% and 11.7% prevalences in cattle, goats and Sheep observed in this study respectively. Other workers have reported higher prevalences of 35.6% in USA, 33.5% in Vietnam, 28.5% in Sirlanka, 47.9% in Spain, 50% in Netherlands and 70% in USA by [33-38] respectively. The relatively high infection rate observed in this study may have resulted from the higher risk of infection reportedly possessed by young animals [21, 28,32,39,40]. The undeveloped immune system and/or the husbandry practice on farms in Nigeria is suggested to facilitate neonatal transmission in which animals of all ages, are grazed together thereby increasing the infection rate [21,41,42].

Our result showed that there was no significant (>0.05) difference between the rate of infection in sheep at Aliero and those at Zuru (P=0.004), while the infection rate between goats at Aliero and Zuru was also not significant in distribution (0.100). However infection amongst cattle in Zuru was significantly higher than in those at Aliero (p=0.004). This finding corroborates a previous report from Iran where significant differences in infection were observed in sheep in six ecological zones [43]

In this study, species-specific prevalence of *Cryptosporidium* was higher in cattle (28.0%) than in Sheep (11.7%) and Goats (17.7%) which is in consonance with the submissions of [9,44,45], who noted that infection is commonly reported in calves than other ruminants, for which reason they have received extensive attention. This study reveals that infection was almost thrice likely in cattle than in sheep and goats (OR=2.510, P=0.0001, CI=1.612 – 3.909).The higher prevalence observed in cattle could be attributed to the intensive or overcrowded management system, where animals of all ages are housed together bringing infected animals together with the healthy ones including young animals whose immune level is low. Animals raised under such confinement will be susceptible to infection due to ease of oocyst contamination and transmission [36,45,46]. Also, the fact that Goats or sheep are not always kept together in such restriction, gives them an advantage of adequate space especially at nights resulting in minimized infection rate among small ruminants.

Several works have indicated that cryptosporidiosis is significantly associated with neonates than adult animals[21,28, ,31,32,36,39,40,47, 48]. But on the contrary, distribution of infection between young and adult sheep in this study was not age related (P=0.100) suggesting a possible interplay of other exposure risks. This trend was also observed by [46] when they reported that there was no significant difference in infection between young and older calves.

Another feature of this study, is the significant difference in infection between male and female cattle (P=0.001). Prevalence was higher in female cattle than in males. The reason for the disparity is not well understood, though this might have resulted from the practice by farmers to retain more females than males for the advantage of breeding and milk production.

The significantly higher rates in diarrhoeic animals recorded in this study, is in line with the submissions of [10, 25,49] reiterating the presence of diarrhoea in young animals as a significant source of oocyst contamination of the environment. Also the environment, management practices, genetics, physiology

and immune status of the animals might have contributed to such outcome. How these factors work is poorly understood and require elucidation.

It has been suggested that the pathogenesis of *Cryptosporidium* infection alongside other enteric infections, such as Rotavirus, Salmonella, Escherichia coli, Eimeria, etc are very likely to result in such diarrhoeic condition [50,51]. Characteristic diarrhoea is thought to result from maldigestion and malabsorption due to reduction in both enzymatic action and absorptive area in the gastrointestinal tract owing to diminution of microvilli and destruction of intestinal epithelia by *Cryptosporidium*. An increase in Paracellular permeability of the intestinal tract and destruction of the functional mucosal barrier system are both as a result of the damage caused by the parasite [52]. In their report, [53] had explained that *C. parvum* infection in calves have shown that jejunum and ileum is mainly affected and the diarrhoea occurs either due to hindrance in sodium absorption coupled with increased prostaglandin production in the intestinal mucosa or owing to increased permeability.

Furthermore, the influence of seasonal variation is likely to affect the incidence of diarrhoea in domestic ruminants. For instance, clean grazing pastures and environments are difficult to maintain by animal owners in Nigeria due to the complex nature of fecal contamination of the environment. There is the possibility of fecal contamination, when rainwater transport *Cryptosporidium* oocysts from faecal deposition in the environment and then into the surface water sources where animals drink freely. This could be one of the factors influencing the numerical increase of oocysts in the environment. The result of which is an increase in the spread of microbes and gastrointestinal parasites associated with diarrhoea.

This study showed that *Cryptosporidium* infection is important in ruminants in Nigeria, particularly calves with female animals at higher risk of infection. The study also underscores the significance of diarrhoea as a key factor in the spread of infection. Thus, given these findings, concerted efforts should be directed towards improving our management systems and diagnosis, in order to ensure healthy production of ruminants and reduce possible zoonotic transmission of the parasite in this and similar settings in Nigeria.

Conflicts of interest: None declared by the authors

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