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Cryptosporidium Infection in Pre-Weaned Ruminants and Pigs in Southwestern Nigeria

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Abstract- The study was carried out to detect Cryptosporidium coproantigens in pre-weaned ruminants and pigs in Ogun State, southwestern Nigeria. Faecal samples of 186 pre-weaned animals comprising calves (n=32), lambs (n=47), goat kids (n=36) and piglets (n=71) were collected and examined for Cryptosporidium antigens by the use of an enzyme-linked immunosorbent assay (ELISA). 60.2% (112/186) of the samples were positive for Cryptosporidium antigens with infection rates of 78.1% (25/32), 51.1% (24/47), 83.3% (30/36) and 46.5% (33/71) for calves, lambs, goat kids and piglets respectively. The infection rates among animal species sampled were significantly different ($p<0.05$) from one another. The rate of infection in neonates, 73.5%, was significantly higher ($p<0.05$) than the rate recorded in other pre-weaned age group (>1 month-3 months). Furthermore, the infection rates, 67.3% and 68.6%, observed in females and diarrhoeic animals respectively were significantly higher ($p<0.05$) than those recorded in males (51.8%) and non-diarrhoeic animals (44.6%). This study demonstrates that Cryptosporidium infection is prevalent among pre-weaned category of ruminants and pigs in Ogun State, Nigeria.

Keywords: cryptosporidium, ELISA, nigeria, pre-weaned animals.

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Cryptosporidium Infection in Pre-Weaned Ruminants and Pigs in Southwestern Nigeria

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Abstract- The study was carried out to detect *Cryptosporidium* coproantigens in pre-weaned ruminants and pigs in Ogun State, southwestern Nigeria. Faecal samples of 186 pre-weaned animals comprising calves (n=32), lambs (n=47), goat kids (n=36) and piglets (n=71) were collected and examined for *Cryptosporidium* antigens by the use of an enzyme-linked immunosorbent assay (ELISA). 60.2% (112/186) of the samples were positive for *Cryptosporidium* antigens with infection rates of 78.1% (25/32), 51.1% (24/47), 83.3% (30/36) and 46.5% (33/71) for calves, lambs, goat kids and piglets respectively. The infection rates among animal species sampled were significantly different ($p<0.05$) from one another. The rate of infection in neonates, 73.5%, was significantly higher ($p<0.05$) than the rate recorded in other pre-weaned age group (>1 month-3 months). Furthermore, the infection rates, 67.3% and 68.6%, observed in females and diarrhoeic animals respectively were significantly higher ($p<0.05$) than those recorded in males (51.8%) and non-diarrhoeic animals (44.6%). This study demonstrates that *Cryptosporidium* infection is prevalent among pre-weaned category of ruminants and pigs in Ogun State, Nigeria.

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I. Introduction

Cryptosporidium is a protozoan parasite that causes enteric infection in several animal species and humans (Quilez et al., 2008). Various species of *Cryptosporidium* infect mammals and humans with *C. parvum* being the most common zoonotic species (Quilez et al., 2008; Chako et al., 2010; Xiao, 2010). While several studies have revealed that cattle are important reservoirs of *C. parvum* (Xiao and Fayer, 2008; Xiao, 2010), sheep have been reported to be more frequently infected by other host-adapted *Cryptosporidium* genotypes, mostly *C. bovis* and the *Cryptosporidium* Cervine genotype and rarely habours the zoonotic *C. parvum* (Elwin and Chalmers et al., 2008; Mueller-Doblies et al., 2008). The most frequently encountered *Cryptosporidium* species in cattle are *C. parvum*, *C. andersoni*, *C. bovis* and *C. ryanae* (Amer et al., 2013; Couto et al., 2014). Sheep and goats are also commonly infected with *C. hominis*, *C. xiaoi*, *C. andersoni*, *C. fayeri*, *Cryptosporidium* Pig genotype II (Diaz et al., 2010; Fiuza et al., 2011). Pigs however are naturally infected with *C. muris*, *C. suis*, *C. srofarum*, *Cryptosporidium* Pig genotype II, *Cryptosporidium* Mouse genotype I and rarely the zoonotic *C. parvum* (Hamnes et al., 2007; Budu-Amoako et al., 2012; Zhang et al., 2013).

The parasite is considered as a major enteric pathogen associated with neonatal diarrhoea in calves (Xiao, 2010; Bhat et al., 2013), lambs and goat kids (Wang et al., 2010; Giadinis et al., 2012; Maurya et al., 2013) and piglets (Maikai et al., 2009; Zhang et al., 2013; Yui et al., 2014). The severity of the diarrhoea seen in cryptosporidiosis in pre-weaned animals is usually high and may ultimately lead to severe loss of condition and death (Ayinmode and Fagbemi, 2010).

The results obtained from several prevalence studies have been largely dependent on the sensitivity and specificity of the screening methods used and the age categories of the animals studied (Brook et al., 2008; Maikai et al., 2009; Ayinmode et al., 2010; Zhang et al., 2013; Yui et al., 2014). The diagnostic methods employed in studies on cryptosporidiosis include microscopic examination of acid-fast stained faecal smears (Maikai et al., 2009; Ayinmode and Fagbemi, 2010), the more sensitive immunoassays such as the enzyme-linked immunosorbent assay and immune-fluorescence assay (Ayinmode and Fagbemi, 2011; Cho et al., 2012; 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 (Ayinmode et al., 2010; Zhang et al., 2013).

This study was conducted to determine the rate of infection of *Cryptosporidium* in pre-weaned age category of ruminants and pigs in a southwestern state of Nigeria by the use of an enzyme-linked immune-sorbent assay.

II. Materials and Methods

a) Sample collection

The study was carried out in Ogun State, southwestern Nigeria. Samples were collected from five farms for each animal species considered. Faecal samples were collected from 186 randomly selected pre-weaned animals comprising calves (n=32), lambs (n=47), goat kids (n=36) and piglets (n=71). Each animal species were categorized into neonates (1 day to 1 month) and other pre-weaned age group (>1 month to 3 months).

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Stool samples were collected directly from the rectum of each animal. For animals 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 faeces without contacting the floor. The faeces were then dropped into individual universal sample bottles and labeled appropriately. The stool samples were conducted to the laboratory in cold packs, where they were catalogued, processed and analyzed. The stool samples were stored under a temperature of 4°C until they were processed.

b) Detection of Cryptosporidium spp. antigens by ELISA

The detection of *Cryptosporidium* spp. coproantigens in the samples was done using a commercially available ELISA kit for faecal samples (RIDASCREEN® *Cryptosporidium*; R-Biopharm AG, Germany). The procedure was carried out according to manufacturer's instructions.

The optical densities (OD) of the samples were read at 450nm using an ELISA reader (BIOTEX; Model: ELx800, Biotex Instruments, USA). Samples were analyzed using the manufacturer's cut-off calculations in the instruction manual. The cut-off was calculated as shown below:

Cut-off = Extinction of the negative control + 0.15

Samples were considered positive if their extinction is more than 10% above the calculated cut off but considered negative if their extinction was more than 10% below the calculated cut-off. Samples were however considered as equivocal and repeated if their extinction was within the range 10% above to 10% below the cut-off.

III. Statistical Analysis

Data was collated and analyzed with Statistical Package for Social Sciences (SPSS) on Windows 7. Chi-square test was used to compare the differences in occurrence of *Cryptosporidium* spp. coproantigens between the categories, sexes and stool consistencies of all animal species at 5% level of significance.

IV. Results

Cryptosporidium spp. antigens were detected in all species of animals studied. 60.2% (112/186) of all pre-weaned animals examined were positive for *Cryptosporidium* coproantigens. The rates of infection were 78.1%, 51.1%, 83.3% and 46.5% for calves, lambs, goat kids and piglets respectively.

The infection rate observed in neonates of all animal species, 73.5% (72/98), was significantly higher ($p<0.05$) than the rate recorded in the other pre-weaned age group (>1 month to 3 months).

The rates of infection in females were higher in lambs, goat kids and piglets while the males recorded higher infection rate in calves. The overall infection rate in females, 67.3%, was significantly higher ($p<0.05$) than the rate observed in the males, 51.8%.

In all the animal species considered, the infection rates were higher in diarrhoeic animals than those without diarrhoea. The occurrence rate of *Cryptosporidium* infection was significantly higher ($p<0.05$) in pre-weaned animals with diarrhoea (68.6%) than the rate observed in those with formed stools (44.6%) (Table 1).

Table 1 : Occurrence of *Cryptosporidium* spp. coproantigens in pre-weaned ruminants and pigs in Ogun State

Animal Species	Infected/Sampled	Occurrence (%)	Age Categories		Gender		Stool Consistency	
			1day -1month	>1month -3months	Male	Female	Diarrhoeic	Non -diarrhoeic
Calves	25/32	78.1	94.4% (17/18)	57.1% (8/14)	82.4% (14/17)	73.3% (11/15)	81.0% (17/21)	72.7% (8/11)
Lambs	24/47	51.1	65.2% (15/23)	37.5% (9/24)	37.5% (9/24)	65.2% (15/23)	62.1% (18/29)	33.3% (6/18)
Goat kids	30/36	83.3	85.0% (17/20)	81.2% (13/16)	66.7% (8/12)	91.7% (22/24)	92.0% (23/25)	63.6% (7/11)
Piglets	33/71	46.5	62.2% (23/37)	29.4% (10/34)	40.6% (13/32)	51.3% (20/39)	54.3% (25/46)	32.0% (8/25)
Total	112/186	60.2	73.5% (72/98)	45.5% (40/88)	51.8% (44/85)	67.3% (68/101)	68.6% (83/121)	44.6% (26/65)

V. Discussion

The detection of *Cryptosporidium* infection in ruminants and pigs within 3 months of age supports several reports carried out in Nigeria (Maikai et al., 2009; Ayinmode and Fagbemi, 2010; Faleke et al., 2013; Pam et al., 2013) and in other countries (Zhang et al., 2013; Yui et al., 2014). Furthermore, the rate of infection (60.2%) observed in this age category of ruminants and pigs was higher than previous reports of 9.3% in Nigeria (Pam et al., 2013), 28.0% in UK (Brook et al., 2008), 24.5% in Australia (Yang et al., 2009) and 30.2% in Egypt (Amer et al., 2010). Differences in prevalence rates of cryptosporidiosis have been suggested to depend on the age category of animals studied, method of detection and season in which sampling was done (Brook et al., 2008; Yang et al., 2009; Yui et al., 2014). The high infection rate observed in this study may therefore result from the higher risk of infection reportedly possessed by pre-weaned animals (Chen et al., 2011; Zhang et al., 2013; Yui et al., 2014) which may be associated with their underdeveloped immune system and/or the husbandry practice on farms in Nigeria in which animals, irrespective of their ages, are grazed together thereby facilitating neonatal transmission of the infection (Ayinmode and Fagbemi, 2010; Fuiza et al., 2011; Bhat et al., 2013). Furthermore, the ELISA used in the study may also contribute to the higher rate of detection of the parasite as this method has been reported to possess higher sensitivity than the acid-fast staining method utilized in majority of previous investigations in Nigeria (Yilmaz et al., 2008).

The specie-specific occurrence rates observed in this study were higher than previous reports of 27.4%, 33.0% and 25.0% among calves in Oyo State (Ayinmode and Fagbemi, 2010), Sokoto State (Faleke et al., 2013) and Sokoto State (Pam et al., 2013) respectively. They were also higher than 16.0% recorded among goat kids in Plateau State (Pam et al., 2013), 15.7% and 13.6% among piglets in Kaduna State (Kwaga et al., 1988; Maikai et al., 2009) and 24.5% among lambs in Australia (Yang et al., 2009). This observation may therefore imply that *Cryptosporidium* oocysts survive for longer periods in the study area, possibly due to the high relative humidity and rainfall, thereby leading to higher risks of transmission to animals (Yu and Seo, 2004).

The lowest occurrence rate recorded among piglets corroborates the submissions of Maddox-Hyttel et al. (2006) and Hamnes et al. (2007) suggesting that *Cryptosporidium*-infected piglets shed lower numbers of oocysts when compared to calves and other animals as these infected piglets shed oocysts intermittently for only 4 weeks.

The higher rate of infection seen in neonates supports the findings of several studies (Maikai et al., 2009; Yang et al., 2009; Ayinmode and Fagbemi, 2010; Budu-Amoako et al., 2012; Zhang et al., 2013). This therefore suggests that neonates of ruminants and pigs are important reservoirs of infection and are mainly responsible for contamination of water with oocysts of *Cryptosporidium*.

Our observation of higher infection in diarrhoeic animals is in tandem with reports of Maurya et al. (2013) and Caccio et al. (2013). The diarrhoea observed in cryptosporidiosis may be associated with the pathogenesis of the parasite and concurrent infection with other enteric pathogens such as *Salmonella*, *Escherichia coli*, *Eimeria*, Rotavirus and *Giardia* (Thompson et al., 2007; Ayinmode and Fagbemi, 2010). This observation therefore suggests that pre-weaned animals having diarrhoea should be routinely screened for *Cryptosporidium*.

The results of this study indicates that *Cryptosporidium* infection is highly prevalent in pre-weaned ruminants and pigs with neonates and diarrhoeic animals being at higher risk of infection and may also serve as important sources of infection to other animals and humans in the study area. The ELISA employed in this study, though rapid, easy to perform and interpret, is less sensitive in detecting the species of *Cryptosporidium* infecting the animals in the study. This has been associated with the cross-reactivity of ELISA-positive *C. parvum* with other species identified by PCR-based molecular techniques (Ayinmode and Fagbemi, 2011). The need to know the species of *Cryptosporidium* affecting pre-weaned ruminants and pigs and their zoonotic potential therefore necessitates the use of molecular techniques in the detection and genotyping of

Cryptosporidium species found in these animals (Amer et al., 2013).

Ethical consideration

The manuscript does not contain clinical studies or patient data.

Conflict of interest

The authors declare that they have no conflict of interest.

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