

Prevalence of *Cryptosporidium parvum* in sheep in Abeokuta, Ogun State, NigeriaAkinkuotu^{1*}, O. A., Fagbemi³, B. O., Jacobs², E. B., Oseni³, S. O. and Adeyanj¹, J.¹Department of Veterinary Microbiology and Parasitology, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria ²Department of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria ³Department of Veterinary Microbiology and Parasitology, University of Ibadan, Ibadan, Oyo State, Nigeria*Corresponding author: divinelivn@yahoo.com; akinkuotuo@funaab.edu.ng**Abstract**

The prevalence of *Cryptosporidium parvum* infection in sheep was determined in Abeokuta, Ogun state, Nigeria. Faeces from randomly selected sheep were analyzed using the Enzyme-linked Immunosorbent assay (ELISA) technique. *Cryptosporidium parvum* antigens were detected in stools of 174 sheep by the use of a commercial ELISA kit. A total of 28.7% of the stools were positive for the antigens. The infection rates decreased with age, the pre-weaned lambs (51.1%) having a significantly higher ($p < 0.05$) rate than the post-weaned lambs (25.5%) and adults (16.7%). The infection rates were also significantly higher ($p < 0.05$) in males (42.9%) and sheep with diarrhoeic stools (41.8%) than in females (17.5%) and sheep with formed stools (17.9%) respectively. This study therefore reveals that the prevalence of *Cryptosporidium parvum* infection is high in sheep reared in Abeokuta and may serve as a reservoir for human infections.

Keywords: *Cryptosporidium parvum*, sheep, faeces, Nigeria.

Introduction

Cryptosporidium species are ubiquitous. They are small protozoan parasites of the phylum Ampicomplexa (Angus, 1990), dwelling in the stomach or small intestine of mammals, birds and man (Thompson *et al.*, 2005), and are among the most common non-bacterial causes of diarrhoea (Current and Garcia, 1991). Recently, there has been increased focus on the parasite as a cause of diarrhoea and reduced weight gain in animals (Olson *et al.*, 2004) and on the importance of animals as potential source of contamination for humans (Fayer, 2004). *Cryptosporidium parvum*, the most important zoonotic *Cryptosporidium* specie (Fayer and Xiao, 2008), have been shown by molecular studies to naturally

infect lambs (Goma *et al.*, 2007). Some studies have however revealed that sheep are more frequently infected with other apparently host-adapted *Cryptosporidium* species/genotypes (Wang *et al.*, 2010) which questions the public health risk of sheep-derived isolates (Elwin and Chalmers, 2008). Additionally, other *Cryptosporidium* species occasionally identified in sheep include *C. hominis*, *C. andersoni*, *C. sius*, *C. fayeri*, *C. bovis*-like genotype (recently named as *C. xiaoi*), *Cryptosporidium* sheep genotype and Pig genotype II (Yang *et al.*, 2009; Wang *et al.*, 2010).

In Nigeria, various studies have been carried out on bovine cryptosporidiosis (Ayeni *et al.*, 1985; Ayinmode and

Fagbemi, 2010). In contrast, fewer studies on cryptosporidiosis in sheep have been reported (Faleke *et al.*, 2006; Pam *et al.*, 2013). In these previous studies the acid-fast staining method was used. This study was therefore designed to determine the prevalence of *Cryptosporidium parvum* in sheep by the use of an Enzyme-linked Immunosorbent Assay, which has been previously reported to be more sensitive than microscopy (El-Shazly *et al.*, 2002).

Materials and methods

Study period and area

The study was carried out in Abeokuta, Ogun State, Nigeria. Ogun state, similar to other southwestern states, is characterized by low temperature, high rainfall and relative humidity throughout the year. The collection of faecal samples was initiated in June, 2012 and ended in November, 2012.

Sample collection

A total of 174 faecal samples were collected from six sheep herds and two sheep markets in the study area. The sheep were grouped into pre-weaned lambs (up to 3 months of age), post-weaned lambs (above 3 months but less than 1 year in age) and adults (1 year and above in age).

A single faecal sample was taken from the rectum of each sheep with a disposable rubber hand glove and emptied into a universal sample bottle and labeled appropriately. The samples were transported, in cold packs, to the laboratory where they were analyzed immediately. If analysis was not done immediately, the samples were preserved at 4°C until they were analyzed.

Detection of Cryptosporidium parvum antigens by ELISA

The detection of *Cryptosporidium parvum* 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 with OD higher than 10% of the cut off (Cut off= 0.185) were reported as positive while those with OD lesser than 10% of the cut off were reported as negative for *Cryptosporidium parvum* coproantigens. Samples with OD between 10% less of the cut off but lower than 10% than cut off are reported as inconclusive and re-examined.

Statistical analysis

Data were analyzed on Statistical Package for Social Sciences (SPSS) on Windows 7. Chi-squared test was used to compare the differences in prevalence of *Cryptosporidium parvum* coproantigens between the age groups, sexes and stool consistencies of sheep at 5% level of significance and 95% confidence interval (CI) (Sari *et al.*, 2009).

Results

The Enzyme-linked Immunosorbent assay revealed that 50 (28.7%) of the samples were positive for *Cryptosporidium parvum* antigens.

The infection rates were observed to decrease with increasing age of sheep with the highest (51.1%) and lowest (16.7%) rates in pre-weaned lambs and adults respectively (Table I). The infection rate in pre-weaned lambs was observed to be significantly higher ($p < 0.05$) than those of the post-weaned lambs ($p = 0.007$; 95% CI= 0.070 to 0.442) and adults ($p < 0.001$; 95% CI= 0.184 to 0.504). The study also showed that the infection rates in males (42.9%) was significantly higher ($p < 0.05$) than 17.5% recorded in the females (Table I) ($p < 0.001$; 95% CI= -0.385 to -0.122).

The infection rate in sheep with diarrhoeic stools (41.8%) was significantly higher ($p < 0.05$) than the 17.9% recorded in those with formed stools (Table I) ($p < 0.001$; 95% CI = -0.371 to -0.107).

The comparison of different ages, sexes and stool consistencies of sheep and their prevalence of *Cryptosporidium parvum* antigens are summarized in Table II.

Discussion

Ovine cryptosporidiosis is largely under-reported in Nigeria (Ayeni *et al.*, 1985; Faleke *et al.*, 2006; Pam *et al.*, 2013).

The 28.7% overall prevalence observed in this study was higher than 1.3% and 16.0% previously reported by Faleke *et al.* (2006) and Pam *et al.* (2013) respectively. This

Table I: Prevalence of *Cryptosporidium parvum* coproantigens in relation to age groups, sexes and stool consistencies of sheep in southwestern Nigeria

Parameters	Number sampled	Number positive (Prevalence)
Age group		
Pre-weaned lambs	47	24 (51.1%)
Post-weaned lambs	55	14 (25.5%)
Adults	72	12 (16.7%)
Sex		
Female	97	17 (17.5%)
Male	77	33 (42.9%)
Stool consistency		
Diarrhoeic	79	33 (41.8%)
Non-diarrhoeic	95	17 (17.9%)
Total	174	50 (28.7%)

Table II: Comparison of the prevalence rates of *Cryptosporidium parvum* coproantigens in different age groups, sexes and stool consistencies of sheep .

Parameters	Prevalence (%)	Odds Ratio (OR)	p-value	95% Confidence Interval	
				Lower limit	Upper limit
Age groups					
Pre-weaned lambs	51.1	3.06	<0.001*	0.184	0.504
Adults	16.7				
Post-weaned lambs	51.1	2.00	0.007*	0.070	0.442
Post-weaned lambs	25.5				
Adults	16.7	1.53	0.227	-0.231	0.055
Sexes					
Female	17.5	2.45	<0.001*	-0.385	-0.122
Male	42.9				
Stool consistencies					
Diarrhoeic	41.8	2.34	<0.001*	-0.371	-0.107
Non-diarrhoeic	17.9				

*Significant difference exists

observation may be due to the higher sensitivity of ELISA in detecting *Cryptosporidium* spp. when compared to the acid-fast staining technique (El-Shazly *et al.*, 2002). It may also be attributed to the ecological and environmental characteristics of the study area which contrasts the low relative humidity and short periods of rainfall of the northern regions of the country in which these previous studies were conducted as supported by Fayer *et al.* (2000) and Yu and Seo (2004) who suggested that high relative humidity and rainfall aid the survival of oocysts in the environment and *vice-versa*. The significantly higher rate of infection observed in pre-weaned lambs (51.1%) corroborates reports of Olson *et al.* (1997), Ulutas and Voyvoda (2004), Yang *et al.* (2009) and Pam *et al.* (2013). This observation may be associated with the underdeveloped immune system of these lambs (Pam *et al.*, 2013) and the husbandry/management system employed in Nigeria in which lambs are housed with adults in a pen thereby facilitating transmission of infection.

The higher prevalence rate recorded in males contrasts the report of Pam *et al.* (2013). The reason for this observation is not known and may require further studies for clarity. It may not be unrelated to contact of males with the perineum of different ewes during mating. *Cryptosporidium* has been reported as a major enteric parasite associated with diarrhoea in sheep (Sari *et al.*, 2009). Furthermore, the diarrhoea observed in cryptosporidiosis may also be due to concurrent infection with other enteric pathogens such as *Giardia*, *Eimeria*, *Salmonella*, rotavirus and various helminthes (Nunez *et al.*, 2003; Ayana *et al.*, 2009). These may account for the significantly higher rate of infection in

sheep with diarrhoeic stools observed in this study. The occurrence of *Cryptosporidium parvum* in asymptomatic sheep implies that apparently healthy sheep can serve as carriers of the infection to other animals and man.

To the best of our knowledge, this is the first report of the use of ELISA in detecting *Cryptosporidium* species in sheep in Nigeria. This study reveals that ovine cryptosporidiosis is of importance and higher in occurrence in Ogun state than some northern states of Nigeria. Molecular characterization of the *Cryptosporidium parvum* in sheep in Nigeria should be carried out to assess the risk of zoonosis they pose.

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