SHORT COMMUNICATION

Sokoto Journal of Veterinary Sciences
(P-ISSN 1595-093X/E-ISSN 2315-6201)
Akinkuotu *et al*/*Sokoto Journal of Veterinary Sciences* (2014) 12(2):52-56.
http://dx.doi.org/10.4314/sokjvs.v12i2.9

Cryptosporidium infection in cattle in Ogun state, Nigeria

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Abstract

The prevalence of *Cryptosporidium* spp. in cattle faeces in Ogun state, Nigeria was determined by a commercially produced enzyme-linked immunosorbent assay kit. Out of a total of 200 samples, 37.5% were positive for *Cryptosporidium* coproantigens. The highest rate of infection (78.1%) was observed in calves up to 3 months of age while adult cattle over 4 years of age had the lowest rate of infection (25.0%). There were significant differences ($p<0.05$) between the infection rates of the different age groups of cattle sampled. There was however no significant difference ($p>0.05$) between the infection rates in males (41.2%) and females (33.6%). Furthermore, the infection rate in diarrhoeic cattle (43.2%) was not significantly higher ($p>0.05$) than in non-diarrhoeic cattle (32.4%). The result of the study showed that the prevalence of cryptosporidiosis is high in cattle in southwestern part of Nigeria with calves being at the highest risk.

Keywords: Cattle, *Cryptosporidium*, ELISA,Ogun state Nigeria.
Received: 01-12-2013 Accepted: 30-06-2014

Introduction

Cryptosporidium is a coccidian parasite found infecting a wide range of mammals (including man), birds and lower vertebrates. Cryptosporidiosis is considered to be of socioeconomic and/or public health importance (OIE, 2004). The infection is self-limiting except in the immunodeficient hosts, such as those with the acquired immunodeficiency syndrome (AIDS) in humans, tuberculosis in cattle and neonates of various animal hosts (Current & Garcia, 1991) in which it may cause protracted diarrhoea.

It is now recognized that *Cryptosporidium* species differ principally in their host range (Adriana *et al*., 2010). While some *Cryptosporidium* species appear to be restricted to particular types of hosts, others have broad host range, including man and are therefore of zoonotic significance (Thompson *et al*., 2008). *Cryptosporidium bovis*, *C. ryanae* and *C. andersoni* are the commonly encountered species affecting cattle in Nigeria (Ayinmode & Fagbemi, 2010; Maikai *et al*., 2011). *Cryptosporidium parvum* is however recognized as an important agent in life-threatening neonatal diarrhoea in calves (Current & Garcia, 1991; Miron *et al*., 1991). Transmission of infection to humans and other mammals is thought to be caused by ingestion of the oocysts of *C. parvum* (Fayer *et al*., 2000). These oocysts are resistant to most common disinfectants and are not readily killed by routine chlorination of water supplies (LeChevallier *et al*., 1991).

Various staining methods have been used in different studies to identify *Cryptosporidium* spp. (Kaur *et al*., 2002; Mahdi & Ali, 2004; Hamedi *et al*., 2005). However, some studies, demonstrated that enzyme-linked immunosorbent assay (ELISA) had higher sensitivity and specificity than the staining methods (Kehl *et al*., 1995; Graczk *et al*., 1996; El-Shazly *et al*., 2002).

In Nigeria, the few available reports on bovine cryptosporidiosis were based on microscopic detection of the oocysts on acid-fast stained faecal smears with reports of prevalence rates of 23.4% in Oyo State (Ayinmode & Fagbemi, 2010), 28.0% in Plateau State (Pam *et al.*, 2013) and 33.0% in Sokoto State (Faleke *et al*., 2014). Fewer reports on the use of immunological methods of detection (especially

ELISA) of *Cryptosporidium* in cattle have been documented (Ayinmode & Fagbemi, 2011) with no published report on Ogun state, southwestern Nigeria. This study therefore was carried out to determine the prevalence of *Cryptosporidium* spp. in cattle in Ogun State using a commercially available *Cryptosporidium*-specific ELISA kit.

Materials and methods

Study area

The study was carried out in Ogun state, southwestern Nigeria. Samples were collected from four cattle farms and two major abattoirs in the four geopolitical zones (Egba, Yewa, Remo and Ijebu) of the state.

Sample collection: A total of 200 faecal samples were collected from different cattle. The cattle were classified on the basis of their age range: 1 day-3 months, >3 months-1 year, >1year- 4 years and > 4years.

A single faecal sample was taken from the rectum of each animal with a disposable rubber hand glove and emptied into a universal sample bottle and labeled appropriately. The samples were thereafter transported, in cold packs, to the laboratory where ELISA was performed on them. If analysis was not to be carried out immediately, the samples were preserved at 4^{o}C for a maximum of 24 hours within which the ELISA was carried out.

Detection of Cryptosporidium antigens by ELISA

The detection of *Cryptosporidium* spp. coproantigens in the samples was done using a commercially available ELISA kit for faecal samples (*Cryptosporidium* (faecal) ELISA kit, Diagnostic Automation Inc., Canada). The procedure carried out was according to manufacturer's instruction.

Briefly, 0.1g of each faecal sample was homogenized in 0.3ml of sample dilution buffer and centrifuged. 100µl of the resulting supernatant was dropped into allocated wells in the microtitre plate and incubated at room temperature for 60 minutes. The plate was thereafter washed with the wash buffer. 100µl of the conjugate was added to each well, incubated for 30 minutes and washed. 100µl of the chromogen was then added to each well and incubated at room temperature for 10 minutes. The reaction was stopped by adding 50µl of stop solution to each well and read using the ELISA reader (BIOTEX; Model: ELx800, Biotex Instruments, USA) at 450nm.

Samples with OD higher than 0.15 were reported as positive while those with OD lesser than 0.15 were reported as negative for *Cryptosporidium* coproantigens.

Statistical analysis

Chi-square test was used to compare the differences in prevalence of *Cryptosporidium* sp. coproantigens between the age groups, sexes and stool consistencies of cattle at 5% level of significance. The analysis was done using the Statistical Package for Social Sciences (SPSS).

Results

Overall prevalence of Cryptosporidium spp.

The enzyme immunoassay showed that 37.5% (75/200) of the samples analyzed were positive for *Cryptosporidium* coproantigens.

Prevalence of Cryptosporidium coproantigens in relation to age of cattle

The highest rate of infection, 78.1% was observed in suckling calves up to 3 months of age. The rate of infection decreased with increasing age of cattle with the lowest rate, 25.0%, being recorded in adult cattle above 4 years of age (Table 1). The infection rate was significantly higher ($p<0.05$) in suckling calves than those above 3 months to 1 year ($p<0.001$; Odds Ratio (OR)=2.15), young adults up to 4 years ($p<0.001$; OR=2.67) and adults above 4 years of age ($p<0.001$; OR=3.12).

Prevalence of Cryptosporidium coproantigens in relation to sex of cattle

Both sexes of cattle were infected with *Cryptosporidium* spp. with males having a higher rate of infection (41.2%) than females (33.6%). However, there was no significant difference ($p>0.05$) between both sexes in their rates of infection (Table 2).

Prevalence of Cryptosporidium coproantigens in relation to stool consistency of cattle

Both symptomatic (diarrhoeic) and asymptomatic (non-diarrhoeic) cattle were positive for *Cryptosporidium* coproantigens. No significant difference ($p>0.05$) was observed between the prevalence of *Cryptosporidium* coproantigens between diarrhoeic (43.2%) and non-diarrhoeic ones (32.4%) (Table 2).

Table 1: Comparison of the infection rates of *Cryptosporidium* coproantigens in different ages of cattle

Age group	Number of cattle (infected/sampled)	Percentage infected	Odds Ratio	p-value
1 day-3 months	25/32	78.1	2.15	<0.001
>3 months-1 year	16/44	36.4		
1 day-3 months	25/32	78.1	2.67	<0.001
>1 year-4 years	21/72	29.2		
1 day-3 months	25/32	78.1	3.12	<0.001
>4years	13/52	25.0		

Table 2: Comparison of the infection rates of *Cryptosporidium* coproantigens between the sexes and stool consistencies of cattle

Parameters		Number of cattle (infected/sampled)	Percentage infected	Odd Ratio (OR)	P-value
Sex	Male	42/102	41.2	1.23	0.199
	Female	33/98	33.6		
Stool consistency	Diarrhoeic	41/95	43.2	1.33	0.117
	Non- diarrhoeic	34/105	32.4		

Discussion

The overall prevalence (37.5%) of *Cryptosporidium* coproantigens observed in this study is consistent with the 32.3% prevalence in Oyo state, Nigeria reported by Ayinmode & Fagbemi (2011) and higher than the reports in other parts of the world: 11% of calves in Sweden (Bjorkman *et al*., 2003), 14% of cows in Denmark (Maddox-Hyttel *et al*., 2006) and 19.7% in Tanzania (Swai *et al*., 2007). This suggests that prevalence of Cryptosporidium is high in cattle reared in southwestern Nigeria. This observation may be associated with the high rainfall and relative humidity characteristic of the southwestern part of the country which favours the survival of the oocysts in the environment.

The observation that suckling calves had a higher rate of infection in this study supports the reports of Fayer *et al*. (1998), Lefay *et al*. (2000), Maddox-Hyttel *et al*, (2006) and Ayinmode & Fagbemi, (2011) who observed that the prevalence of *Cryptosporidium* spp. in pre-weaned calves is usually high. This may be attributed to higher susceptibility of calves to infection due to lack of previous exposure to the parasite. It may also suggest that the management practices by pastoralists in Nigeria, where calves are grazed with the adults, may increase their risk of infection by consumption of contaminated feed and water by the calves (Ayinmode & Fagbemi, 2010). An acquired immunity towards *Cryptosporidium* in adults due to infections in early life (Harp *et al*., 1990) may account for the low incidence of clinical condition and thus the low shedding of *Cryptosporidium* oocysts in this age group. The high rate of infection in calves may also be of zoonotic concern as calves have been reported to be important reservoirs of the infection (Barrington *et al*., 2002; Mallon *et al*., 2003a; Mallon *et al*., 2003b; Santin *et al*., 2004; Fayer *et al*., 2007; Xiao, 2010), serving as important source of contamination of water with *C. parvum* oocysts (Xiao, 2010).

The high rate of infection observed in male cattle is in tandem with reports of Maikai *et al*. (2011) but contrary to the findings of Ayinmode & Fagbemi (2010). The reason for this observation is not known. Further research is required to elucidate this and other possible reasons for the differences in infection rates between the sexes of cattle.

The lack of significant association between diarrhoea and presence of *Cryptosporidium* coproantigens observed in this study supports a similar observation by Ayinmode & Fagbemi (2010). This may be attributed to subclinical chronic phases of infection with the parasite which may not have the clinical sign of diarrhoea in the affected cattle (Ralston *et al*., 2003; Olson *et al*., 2004; Fayer *et al*., 2006).

The ELISA employed in this study, although reported to be highly sensitive in the detection of *Cryptosporidium* spp. (El-Shazly *et al*., 2002), have the risk of producing false positive results. It is therefore important to utilize molecular techniques to detect and characterize the *Cryptosporidium* spp. found in cattle in the study area as this method has

been reported to possess very high sensitivity and specificity when compared to the ELISA and microscopy (Ayinmode & Fagbemi, 2011). This study thus indicates that cryptosporidiosis is common in cattle in the southwestern part of Nigeria with suckling calves being at a higher risk of infection than older ones.

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