

## Prevalence of *Cryptosporidium* infection in cattle from South Western Nigeria

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### ABSTRACT

This study investigates the prevalence of *Cryptosporidium* sp. infection in Oyo state, south western Nigeria. Fecal specimens from 406 cattle were randomly collected and examined for the presence of *Cryptosporidium* sp. oocysts using the formol ethyl ether concentration and the modified Kinyoun acid-fast staining method. The results showed that the overall prevalence of infection was 23.4% (95/406), with an infection rate of 27.4%, 28.1% and 19.9% in cattle less than 6 months, 7-12 months and over 12 months respectively. Although no significant difference ( $P>0.05$ ) exists between the age groups, calves less than 6 months of age are more likely to be infected than adults (OR: 1.512; 95%CI: 0.849-2.709). A significant difference ( $P<0.0001$ ) also existed between the infection rates in female and male cattle, with the female cattle being twice as likely to be infected (OR: 2.847; 95%CI: 1.755-4.618). The infection rate was 84.2% (80/95) in non-diarrheic cattle and 15.8% (15/95) in the diarrheic ones. No significant association was observed between diarrhea and the presence of *Cryptosporidium* sp. oocysts ( $P = 0.9468$ ; OR: 0.979; 95%CI: 0.522-1.636). The results indicate that *Cryptosporidium* sp. infection is prevalent in Nigeria, especially in asymptomatic cattle that could serve as reservoirs for the zoonotic infection in humans.

**Key words:** *Cryptosporidium* sp., cattle, oocyst, diarrhea, prevalence, Nigeria

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### Introduction

*Cryptosporidium* is one of the most common enteropathogens causing diarrhea in humans and domestic animals, including livestock, dogs, cats and wildlife (FAYER, 2004). Both the prevalence and the severity of infection increase in immunodeficient animals, especially in neonates of some animal species such as ruminants. However, the infection is self-limiting in healthy or immunocompetent individuals (CURRENT et al., 1983).

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In cattle, *Cryptosporidium parvum* is considered to be the most common enteroparasite in calves during the first week of life, frequently as a coincidental infection with other viral, bacterial and parasitic pathogens (FAYER et al., 1998; DE GRAAF et al., 1999; O'HANDLEY et al., 1999). *Cryptosporidium* infection can negatively influence growth rate, feed conversion and milk production (ESTEBAN and ANDERSON, 1995) leading to a drop in the economic benefits derived from livestock production.

Cattle have been implicated as a major source of *C. parvum* in pasture run off, which is responsible for environmental contamination and human infection either by direct or indirect contact through fecal contamination of food or water for human consumption (CURRENT and GARCIA, 1991).

Bovine cryptosporidiosis is widespread and studies have shown a wide range of oocyst shedding dynamics depending on the age, clinical situation and breeding system of the animals (MALDONADO-CAMARGO et al., 1998). Although cattle are the most common domestic animals and the major meat-producing animals in Nigeria with economic value, there is no readily available data on cryptosporidiosis in cattle. Cattle are brought in close proximity to human sources of water and farms during grazing, due to management systems that are mainly semi-intensive. The potential hazard that *C. parvum* from cattle poses to public health in Nigeria, therefore, makes it necessary to investigate the prevalence of bovine cryptosporidiosis and also understand the factors that lead to the transmission and spread of infection in animals.

This study is aimed at investigating the prevalence of *Cryptosporidium* infection in cattle in Oyo state of southwestern Nigeria, with the opinion that this knowledge will serve as a basis for screening the *Cryptosporidium* species and genotypes in Nigerian cattle and also facilitate further studies on the possibility of zoonotic transmission of the disease from animals to humans.

### **Materials and methods**

*Fecal sampling.* Stool specimens were collected during the dry season from 406 cattle randomly selected from farm settlements in Oyo state (south-western) of Nigeria. The sample size was determined using Win episode software (ORTEGA et al., 1996), taking an expected prevalence of 50% (number recommended when disease frequency is unknown) (THRUSFIELD, 1995), a confidence interval of 95% and an error of 5% with an estimated cattle population of 3 million.

Animals were classified on the basis of their age range: less than 6 months of age (84), between 7-12 months (96) and greater than 1 year old (226).

A single fecal sample was taken from the rectum of each animal by means of a disposable plastic bag and emptied into a wide-mouthed disposable plastic container. The

fecal samples were transported to the laboratory and preserved in 75% alcohol at room temperature until processed (JONGWUTIWES et al., 2002).

*Cryptosporidium* oocyst detection technique. Fecal specimens were concentrated by the formalin-ethyl acetate sedimentation method (GARCIA and SHIMIZER, 1981). Briefly, 5 mL of the formalin-treated stool specimen was washed in 10% Formalin-saline, and the sediment, collected by centrifugation at  $650 \times g$  for 5 min, was suspended in 8 mL of formalin-saline in 3 mL of ethyl acetate. This mixture was mixed thoroughly for 3 min and centrifuged at  $500 \times g$  for 5 min, resulting in four layers: a layer of ethyl acetate, a plug of debris, a layer of formalin-saline, and the sediment. The plug was rimmed with an applicator stick and the top three layers were decanted. One portion of the sediment was placed on a microscope slide and dried for the acid-fast stain.

Smears of sediment were stained by the modified Kinyoun acid-fast stain (GARCIA and SHIMIZER, 1981). The smears were fixed in methanol for 1 min, stained in the primary dye (Kinyoun carbolfuschin) for 15 min, and then decolorized for 1 min in 10%  $H_2SO_4$ . The smear was counterstained with Malachite green.

The intensity of infection was estimated semi-quantitatively according to the average number of oocysts in 20 randomly selected fields at  $100 \times$  magnification. A sample is negative if there are no oocysts, slightly positive if there are 1-5 oocysts; moderately positive if there are 6-10 oocysts and highly positive if more than 10 oocysts are seen (MA and SOAVE, 1983; GARCIA, 2001).

*Statistical analysis.* Data were computed using prisms (statistical) software. A Chi-squared test was used to compare the differences in prevalence of *Cryptosporidium* oocysts between age-groups of cattle at a 5% level of significance. The 95% confidence intervals on overall prevalence were also computed.

## Results

*Prevalence of Cryptosporidium* sp. oocysts. The prevalence results of *Cryptosporidium* sp. oocysts among the age groups are presented in Table 1. Analysis showed an overall prevalence of 23.4% in the cattle examined with a modified Kinyoun acid staining method.

Table 1. Prevalence of *Cryptosporidium* sp. oocysts in cattle, with age group

Age range	No. positive/ No. examined	Infection rate (%)	95% CI	No. of infected with diarrhea
< 6 months	23/84	27.4	0.1765-0.3712	5 (21.7%)
7 - 12 months	27/96	28.1	0.1897-0.3712	7 (24.9%)
> 12 months	45/226	19.9	0.1467-0.2516	3 (6.7%)
Total	95/406	23.4		15 (15.8%)

Out of the fecal samples surveyed from different farms in Oyo state, 23.4% (95/406) were positive and 76.6% (311/406) were negative for *Cryptosporidium* oocysts. The infection rates with *Cryptosporidium* sp. were highest (28.1%; 27/96) in calves between 7-12 months, followed by calves that were less than 6 months of age (27.3%; 23/84), while the adult cattle (>12 months) had the least infection rate of 19.9% (45/226).

The infection rate of *Cryptosporidium* sp. amongst adults (>12 months) was not significantly different ( $P>0.05$ ) from those less than 6 months of age. However, statistical analysis showed that calves less than 6 months of age are more likely to be infected than the adults (OR: 1.512; 95%CI: 0.849-2.709).

Microscopic examination showed that cattle of both sexes were infected with *Cryptosporidium* sp. as 38.1% (43/113) female and 17.7% (52/293) male animals were infected.

The evaluation of the consistency of the stool samples collected during the survey revealed that only 16% (65/406) were diarrheic while 84% (341/406) were firm. Out of the 95 positive samples, 15.8% (15/95) were found to be diarrheic while the remaining 84.2% (80/95) positive samples were non-diarrheic. The results of age, diarrhea and sex associated with positivity to cryptosporidiosis are summarized in Table 2.

Table 2. Results of age, diarrheic status and sex associated with *Cryptosporidium* sp. oocysts in fecal samples after modified acid fast staining

	No. infected/ No. studied	Percentage (%)	Odd ratio (OR)	95% CI	P-value
<b>Age</b>					
<6 months	23/84	27.4	0.960	0.051-1.854	0.0912
7-12 months	27/96	28.1			
<6 months	23/84	27.4	1.512	0.849-2.709	0.1588
>12 months	45/226	19.9			
<b>Diarrhea</b>					
Diarrheic	15/65	23.1	0.979	0.522-1.636	0.9468
Non-diarrheic	80/341	23.5			
<b>Sex</b>					
Female	43/113	38.1	2.847	1.755-4.618	$P<0.0001$
Male	52/293	17.7			

### Discussion

The overall prevalence (23.4%) of *Cryptosporidium* in cattle obtained in this study is consistent with that observed in studies from other parts of the world: 19% of calves in Spain (PANCIERA et al., 1971), 20% of calves in Canada (O'DONOGHUE, 1995); 19-36%

of cattle in Germany (JOACHIM et al., 2003); and 35% of calves in the United States of America (SANTIN et al., 2004). The result of this study thus implies that *Cryptosporidium* infection in cattle, as well as the possible risk of transmission to humans by cattle, is important in Nigeria as it is elsewhere in the world. Hence, considerable attention should be paid to preventing the spread of the infection.

Our findings showed that there was no significant difference ( $P > 0.05$ ) between the rate of the infection in calves less than 6 months and those between 7-12 months of age ( $P = 0.0912$ ), while the infection rate amongst adults ( $>12$  months) was also not significantly different ( $P > 0.05$ ) from those less than 6 months of age ( $P = 0.1588$ ). However, calves less than 6 months old are more likely to be infected than the adults (OR: 1.512; 95%CI: 0.849-2.709). This information corroborates a previous report that showed that the prevalence of Cryptosporidiosis is higher in calves than in adult cattle (CURRENT and GARCIA, 1991). Whereas most published studies indicate that the prevalence was significantly higher in newborn and suckling calves (LORENZO-LORENZO et al., 1993; SCOTT et al., 1994; QUILEZ et al., 1996; LEFAY et al., 2000; CASTRO-HERMIDA et al., 2002), this study on the contrary showed no significant difference ( $P > 0.05$ ) between the infection rates of all ages of calves (one day old to 7 months). This observation could be attributed to the unique management systems employed by pastoralists in Nigeria, where young calves, irrespective of their age, are raised together with their parents under the same field conditions, in contrast to what is usual in developed nations where the calves are isolated from the dam. Calves raised in such confinement were found to be more susceptible to *Cryptosporidium* infection due to poor immunity and ease of oocyst contamination through bucket feeding (HARP et al., 1990; McCLUSKEY et al., 1995; CASTRO-HERMIDA et al., 2002). Hence, factors other than age may play an important role in determining the susceptibility of calves to Cryptosporidiosis.

This study also reports a significant difference ( $P < 0.0001$ ) between the infection rates of *Cryptosporidium* sp. in female and male cattle, with the female animals twice as likely to be at risk than the males (OR: 2.847; 95%CI: 1.755-4.618). The reason for this disparity is not known, although this outcome could be attributed to the usual practice of having a higher female:male ratio in a herd and also the retention of female animals for breeding and milk production. It could also be related to host intrinsic factors (genetics, physiology and immunology) and extrinsic factors (environment and management practices). The underlying mechanisms of the effect of these factors are poorly understood and require clarification.

*Cryptosporidium* sp. has been highly associated with diarrhea especially in calves (QUILEZ et al., 1996; ANDERSON, 1998; DE GRAAF et al., 1999). However, the current study showed that there is no significant association between diarrhea and the presence of *Cryptosporidium* oocysts (OR: 0.979; 95%CI: 0.522-1.636). This supports the

observations of SNODGRASS et al. (1986), KAMINJOLO et al. (1993), O'HANDLEY et al. (1999) and BJORKMAN et al. (2003) who suggested that *Cryptosporidium parvum* may often be identified in non-diarrheic calves. Other findings suggested that diarrhea in *Cryptosporidium* infection may be the result of other enteropathogens that are usually associated with the infection (ANDERSON, 1998; DE GRAAF et al., 1999).

Furthermore, seasonal variations may also affect the incidence of diarrhea in the herd. For instance, the maintenance of clean grazing pastures and environment by pastoralists in Nigeria is usually more difficult during the rainy season than in the dry season due to the complexity of environmental contamination with fecal samples, which usually encourages an increase in concurrent infection by microorganisms and gastrointestinal parasites associated with diarrhea in calves and adult cattle. The influence of seasonal variations in the prevalence of *Cryptosporidium* sp. will be explored in a future study.

The result of this study corroborates an earlier study which suggested that the severity of cryptosporidiosis is influenced by factors such as the infectious dose, herd immunity, the presence of concurrent infections, nutrition and husbandry (OLSON et al., 2004).

Furthermore, the sub-clinical nature of the infection could also be a reason why there may not always be a significant association between diarrhea and the presence of *Cryptosporidium* sp. infection. Besides, an unidentified *Cryptosporidium* species could be responsible for the infection since there is evidence to show that apart from *C. parvum* infection in cattle, *C. andersoni* has also been implicated in bovine cryptosporidiosis (RALSTON et al., 2003; FAYER et al., 2006; SANTIN et al., 2004) which could manifest as a chronic infection without noticeable clinical signs (ANDERSON, 1998; OLSON et al., 2004).

This study showed that *Cryptosporidium* infection is important in Nigerian cattle, particularly in calves, with female cattle at higher risk of infection than males. This work also suggests that the presence of diarrhea in *Cryptosporidium* infection may be more from the interplay of other factors (like concurrent infection, seasonal variation, management and others) than the sole impact of *Cryptosporidium* sp. This result agrees with SNODGRASS et al. (1986) and KAMINJOLO et al. (1993) who also found no statistically significant association between infection and diarrhea. Thus, asymptomatic cattle (without diarrhea) can act as reservoirs for *Cryptosporidium* in man and animals.

Giving these findings, deliberate efforts should be directed towards the diagnosis and prevention of Cryptosporidiosis in Nigeria so as to prevent the zoonotic transmission of the disease.

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**AYINMODE, A. B., B. O. FAGBEMI: Prevalencija invazije vrstama roda *Cryptosporidium* u goveda na području Jugozapadne Nigerije. Vet. arhiv 80, 723-731, 2010.**

**SAŽETAK**

U radu je prikazano istraživanje prevalencije invazije protozoima roda *Cryptosporidium* u državi Oyo u Jugozapadnoj Nigeriji. Uzorci izmeta 406 goveda bili su nasumce prikupljeni i pretraženi na prisutnost oocista metodom koncentracije u formoletileteru i bojenjem po Kinyounu na acidorezistentne organizme. Dokazana je prevalencija od 23,4% (95/406). U goveda mladih od šest mjeseci prevalencija je iznosila 27,4%. U goveda u dobi od sedam do dvanaest mjeseci prevalencija je iznosila 28,1%, a u starijih od dvanaest mjeseci iznosila je 19,9%. Iako nije dokazana značajna razlika ( $P>0,05$ ) među skupinama različite dobi, smatra se da je telad mlađa od šest mjeseci primljivija na invaziju od odraslih. Značajna je razlika ( $P<0,0001$ ) dokazana između prevalencije u goveda različitoga spola. U krava je dokazana dvostruko veća mogućnost invazije. Stopa invadiranosti iznosila je 84,2% (80/95) u goveda bez proljeva, a 15,8% (15/95) u goveda s proljevom. Nije ustanovljena povezanost između pojave proljeva i prisutnosti oocista kriptosporidija ( $P = 0,9468$ ; OR: 0,979; 95%CI: 0,522-1,636). Rezultati pokazuju da je invazija protozoima *Cryptosporidium* sp. česta u Nigeriji, osobito u goveda koja ne pokazuju kliničke znakove bolesti, a mogu biti znatan izvor invazije za ljude.

**Ključne riječi:** *Cryptosporidium* sp., govedo, oocista, proljev, prevalencija, Nigerija

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