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Occurrence of *Cryptosporidium* species coproantigens on a University teaching farm in Nigeria

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Abstract

This study was carried out to assess the potential of animals, used for teaching and research, as a source of *Cryptosporidium* infection for students and staff of a University in Nigeria. Faecal samples from 185 animals reared on the teaching and research farm were collected and examined for *Cryptosporidium* spp. antigens by the use of an enzyme-linked immunosorbent assay (ELISA). From all the samples evaluated, 35.7% (66/185) were positive for *Cryptosporidium* spp. antigens with an infection rate of 30.6% (15/49), 40.7% (22/54), 43.9% (18/41) and 26.8% (11/41) for cattle, sheep, goats and pigs respectively. The rate of infection was significantly higher ($p<0.05$) in pre-weaned animals (63.6%) than in the post-weaned (23.6%) and adult (29.1%) animals. The infection rates, 54.8% and 42.3%, for diarrhoeic and female animals were significantly higher ($p<0.05$) than in non-diarrhoeic and male animals respectively. The presence of coproantigens of *Cryptosporidium* spp. observed in stool samples of ruminants and pigs suggests that these animals may be considered as a potential reservoir of this protozoa, that is able to contaminate the environment, infect other domestic and wild animals and in some cases humans.

Keywords: *Cryptosporidium*, ELISA, Nigeria, Pigs, Ruminants
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Introduction

Cryptosporidium is a protozoan parasite that causes enteric infection in many species of mammals, including humans (Quilez *et al*., 2008). Various studies have revealed that ruminants are important reservoirs of this parasite (Quilez *et al*., 2008). The most encountered *Cryptosporidium* species in cattle are *C. parvum*, *C. andersoni*, *C. bovis* and *C. ryanae* (Xiao *et al*., 2012; Amer *et al*., 2013; Couto *et al*., 2014). In contrast to the vast studies on bovine cryptosporidiosis, the occurrence of the disease in small ruminants has received little attention. The parasite is however considered as one of the major enteric pathogens associated with neonatal diarrhoea and mortality in sheep and goats (Wang *et al*., 2010; Maurya *et al.,* 2013).

Various studies have identified several *Cryptosporidium* species in pigs (Yatswako *et al*., 2007; Maikai *et al*., 2009; Budu-Amoako *et al.,* 2012; Zhang *et al*., 2013; Yui *et al*., 2014). Recent genetic characterization studies have revealed that pigs are infected with a genetically distinct and apparently host-adapted species of *Cryptosporidium* (*Cryptosporidium* "pig" genotype II) and *C. suis*. They can also be naturally infected with *C. muris*, *Cryptosporidium* mouse genotype I and the zoonotic *C. parvum* (Budu-Amoako *et al.,* 2012).

The role that various livestock play in the foodborne and waterborne transmission of *Cryptosporidium* to other animals and humans has been thoroughly investigated by various researchers (Uehlinger *et al*., 2006; Zintl *et al*., 2007; Coklin *et al*., 2009; Ryan & Xiao, 2009; Fayer *et al*., 2010).

Molecular studies have revealed that *C. parvum* infection occurs in all mammals and poses the highest risk of zoonotic transmission (Feng *et al*., 2007; Goma *et al*., 2007). Some studies have

however revealed that sheep are more frequently infected by other apparently host-adapted *Cryptosporidium* genotypes, mostly *C. bovis* and the *Cryptosporidium* Cervine genotype, which questions the public health risk of sheep-derived isolates (Elwin & Chalmers, 2008; Mueller-Doblies *et al*., 2008). Other *Cryptosporidium* species that affect sheep and goats are *C. parvum*, *C. hominis*, *C. xiaoi*, *C. andersoni*, *C. fayeri* and *Cryptosporidium* pig genotype II (Diaz *et al*., 2010; Wang *et al*., 2010, Fiuza *et al*., 2011).

The diagnosis of cryptosporidiosis is usually achieved by conventional microscopic examination of acid-fast stained faecal smears. The need for rapid and cost-effective diagnosis has led to the development of immunoassay techniques like immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA). These methods are relatively easy to perform and interpret (Ayinmode & Fagbemi, 2011; Cho *et al*., 2012; Giadinis *et al*., 2012). However, neither microscopy nor ELISA can differentiate *Cryptosporidium* species and genotypes. *Cryptosporidium* species can only be identified by Polymerase Chain Reaction (PCR)-based techniques (Monis and Thompson, 2003; Ayinmode and Fagbemi, 2011; Giardinis *et al.,* 2012).

In academic institutions, such as veterinary and animal-based colleges, where students and staff are in frequent contact with ruminants and pigs, the risk of humans contracting cryptosporidiosis may be high. A similar study carried out at the Atlantic Veterinary College bovine teaching herd, University of Prince Edward Islands, Canada detected no *Cryptosporidium* species in cows used for teaching and research (Uehlinger *et al*., 2006).

In this present study, we determined the occurrence of *Cryptosporidium* spp. coproantigens in cattle, sheep, goats and pigs used for academic purposes at a teaching and research farm of a Nigerian University with respect to the potential health concern for students and staff in regular contact with these animals.

Materials and methods

Herd and husbandry

Diarrhoeic and non-diarrhoeic cattle (n=49), sheep (n=54), goats (n=41) and pigs (n=41) of different ages were randomly sampled from herd sizes of 89, 95, 92 and 84 respectively. Each animal species sampled were divided into pre-weaned (up to 3months), post-weaned (>3months to 1year) and adults (>1 year) age categories. These animals are used for teaching and research purposes for undergraduate and postgraduate students and staff of the veterinary and animal science-based colleges at the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. These animals were born and raised on the farm or acquired from different ruminant and pig markets in and around the state.

Teaching procedures performed on these animals include restraints, blood and faecal collection, rectal palpation, semen collection, clinical examination, various surgical procedures and treatment of disease conditions.

The different species of animals are housed in separate areas on the farm. The ruminants are managed semi-intensively while the pigs are reared intensively. Different breeds and age groups of cattle are housed together in large pens, similar to the housing provided for sheep and goats. The pens are cleaned twice a day.

Sampling

The study was carried out between July, 2012 and February, 2013. 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^{o}C until they were processed.

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.

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 animals at 5% level of significance.

Results

Cryptosporidium spp. antigens were detected in all species of animals examined. A total of 35.7% (66/185) of the animals were positive for *Cryptosporidium* spp. antigens. The rates of infection were 30.6%, 40.7%, 43.7% and 26.8% for cattle, sheep, goats and pigs respectively (Table 1).

Antigens of *Cryptosporidium* spp. were detected in all categories of the animal species sampled with the exception of post-weaned piglets in which no parasite was detected. The highest rates of infection of *Cryptosporidium* spp. were observed in the pre-weaned category of all animal species. A significant difference ($p<0.05$) was observed in the infection rate observed between pre-weaned animals, 63.6% (24/44) and other age groups (Table 1).

The occurrence of *Cryptosporidium* spp. antigens was significantly higher ($p<0.05$) in diarrhoeic, 54.8% (40/73) and (22/81) respectively (Table 1), female animals, 42.3% (44/104) than in non-diarrhoeic, 23.2% (26/112) and male animals, 27.2%.

Table 1: Occurrence of *Cryptosporidium* spp. coproantigens in a university farm

Animal Species	Infected/Sampled	Occurrence (%)	Categories			Gender		Stool Consistency	
			Pre-weaned	Post-weaned	Adults	Male	Female	Diarrhoeic	Non-diarrhoeic
Cattle	15/49	30.6	50.0% (4/8)	16.7% (2/12)	31.0% (9/29)	15.0% (3/20)	41.4% (12/29)	39.1% (9/23)	23.1% (6/26)
Sheep	22/54	40.7	69.2% (9/13)	42.9% (6/14)	25.9% (7/27)	30.4% (7/23)	48.4% (15/31)	55.0% (11/20)	32.4% (11/34)
Goat	18/41	43.7	75.0% (9/12)	33.3% (5/15)	28.6% (4/14)	33.3% (6/18)	52.2% (12/23)	85.7% (12/14)	22.2% (6/27)
Pig	11/41	26.8	54.5% (6/11)	0.0% (0/14)	31.2% (5/16)	30.0% (6/20)	23.8% (5/21)	50.0% (8/16)	12.0% (3/25)
Total	66/185	35.7	63.6% (28/44)	23.6% (13/55)	29.1% (25/86)	27.2% (22/81)	42.3% (44/104)	54.8% (40/73)	23.2% (26/172)

Discussion

This study is similar to a previous study conducted by Uehlinger *et al*. (2006) at the Atlantic Veterinary College teaching herd, University of Prince Edward Islands, Canada which investigated the occurrence of *Giardia duodenalis* and *Cryptosporidium* species. To our knowledge, this is the first study to report the occurrence of *C. parvum* in ruminants and pigs used for teaching and research in Nigeria.

The conditions on the University farm will facilitate close, regular and repeated contact of humans with animals, thereby potentially putting students and staff at risk of contracting various zoonotic diseases such as *Cryptosporidium*, therefore supporting the observations of Robertson *et al*. (2010) and Ayinmode *et al*. (2012).

The detection of *C. parvum* coproantigens in ruminants and pigs on the farm contrasts the reports of Uehlinger *et al*. (2006) who reported no *Cryptosporidium* infection in adult cattle in a Veterinary college teaching herd. The detection of the parasite in this study therefore indicates that the animals used for teaching and research in Nigeria may serve as sources of infection of *Cryptosporidium* to animal handlers. The high infection rate recorded in the pre-weaned category of animals supports the reports of Xiao (2010), Budu-Amoako *et al*. (2012), Zhang *et al*. (2013) and Yui *et al*. (2014) and this may imply that neonates of these animals are highly susceptible to *Cryptosporidium* infection, majorly due to their underdeveloped immune system and/or the husbandry practice on the farm in which animals, irrespective of the age, are grazed together thereby facilitating the infection of the neonates (Ayinmode & Fagbemi, 2010; Fiuza *et al*., 2011; Bhat

et al., 2013). Numerous studies have revealed that *Cryptosporidium* can be detected in both symptomatic and apparently healthy animals (Maddox-Hyttel *et al*., 2006; Maikai *et al*., 2009; Ayinmode & Fagbemi, 2010).The high detection rate in animals with diarrhoea observed in this study corroborates several reports (Maddox-Hyttel *et al*., 2006; Maikai *et al*., 2009) which have attributed it to the pathogenesis of the parasite and concurrent infection with other enteric pathogens such as *Salmonella*, *Escherichia coli*, *Eimeria*, Rotavirus and *Giardia* (Ayinmode & Fagbemi, 2010). This observation may also indicate that diarrhoeic animals on the University farm may contribute to the spread of high numbers of *Cryptosporidium* oocysts in the University environment and also serve as a source of infection of water used on the farm (Harith *et al.,* 2012; Maurya *et al*., 2013).

The ELISA employed in this study was indeed rapid, easy to perform and interpret, there was, however, the possibility of false-positive detections resulting from the ability of the ELISA to detect products of disintegrated organisms or antigens from extracellular life cycle stage or cross react with other Ampicomplexan parasites (Ayimode & Fagbemi, 2010, 2011; Cho *et al*., 2012). The ELISA is however a less sensitive method in detecting the species of *Cryptosporidium* infecting the animals in the study. This is associated with the cross reactivity with other *Cryptosporidium* species identified by PCR-based molecular techniques as reported by Ayinmode & Fagbemi (2011).

Previous reports revealed that not all *C. parvum* isolates are infective to humans as some of these isolates have been found to be host-adapted (Alves *et al*., 2006; Xiao *et al*., 2006; Couto *et al.,* 2014), which reiterates the need to know the species of *Cryptosporidium* affecting the herd and their zoonotic potential therefore necessitating the use of molecular techniques in the detection, genotyping and characterization of the *Cryptosporidium* species found in these animals (Amer *et al*., 2013).

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