

PREVALENCE OF *cryptosporidium* PARASITES IN CATTLE LIVING IN THREE COMMUNITIES IN THE SOUTHERN PART OF GHANA

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Abstract

Cryptosporidium is a protozoan that causes gastro-intestinal illness with diarrhoea in both humans and other vertebrates. *Cryptosporidium* parasites, some of which are zoonotic have cattle as a reservoir hosts. Nonetheless, the contribution of cattle in the epidemiology of cryptosporidiosis in Ghana is under estimated. This is because most studies on cryptosporidiosis in Ghana have focused on morphological identification of the oocysts in children, HIV-AIDS patients and water bodies. In this study, the prevalence of *Cryptosporidium* parasites from cattle living in three communities, Agona Nantifa, Osamkrom and Belekope in southern Ghana was investigated. A total of 242 farm animals from five farms were screened for the presence of *Cryptosporidium* oocyst by the use of compound light microscope after employing Ziehl-Neelsen acid-fast staining technique. The small-subunit rRNA was amplified by polymerase chain reaction to identify the *Cryptosporidium* parasite. Majority (92.15%) of the farm animals screened did not present diarrheic faeces at the time of sample collection. No *Cryptosporidium* oocyst was morphologically identified in any of the samples using the compound light microscope. However, five faecal samples were positive for *Cryptosporidium* parasite, representing prevalence of 2.07%. All positive samples were from pre-weaned calves with diarrhoea from Belekope.

Keywords

Cryptosporidium parasites, cattle, Formol-ether concentration, PCR, Ghana

Introduction

Cryptosporidium is an obligate intracellular parasite responsible for one of the most common waterborne diseases throughout the world today. Even though the disease is self-limiting in immune-competent individuals, cryptosporidiosis could be severe and life threatening in children as well as persons with compromised immune system (Abubakar et al., 2007). In developing countries, cryptosporidiosis is most prevalent during early childhood, with as many as 45% of children experiencing the disease before the age of 2 years (Mor and Tzipori, 2008). Infected hosts shed oocysts, which is the environmentally resistant transmission stage of the parasite within their faeces (Fayer et al., 1997; Fayer and Ungar, 1986). These oocysts are immediately infectious and may remain in the environment for very long periods without losing their infectivity. As a result of its robust wall, oocysts are resistant to physical and chemical exposures such as the normal dosage of chlorine used in water treatment (Korich et al., 1990). Moreover, *Cryptosporidium* oocyst is seldom removed during filtration in water treatment as a result of its relatively small size of about 4-6 μm in diameter (World Organisation for Animal Health (OIE), 2008).

Over 26 species of *Cryptosporidium* parasite have been identified, infecting hosts such as fishes, amphibians, reptiles, birds and mammals (Fayer et al., 1997; Ryan et al., 2014). While some of the parasites are host specific, others have

multiple hosts. For instance *Cryptosporidium parvum*, the primary species that infect humans have also been isolated from over 150 species of mammals (Fayer et al., 2000). Cattle has been important source of zoonotic cryptosporidiosis since the 1980s (Castro-Hermida et al., 2007; Fayer et al., 2000; Geurden et al., 2008; Paoletti et al., 2009). The main mode of transmission of this zoonotic pathogen is through ingesting a stable and robust oocyst from the environment. Risk of human infection has been based on physical contact with cattle, contamination of fresh fruits and vegetables with manure, and runoffs from farms into drinking water supplies. For instance, contact with infected calves has been implicated as the cause of many cryptosporidiosis outbreaks in veterinary students, research technicians and children attending agricultural camps and fairs (Kiang et al., 2006; Preiser et al., 2003; Smith et al., 2004).

In Ghana, studies on cryptosporidiosis have mainly centred on morphological identification of the oocysts in children (Addy and Aikins-Bekoe, 1986; Adjei et al., 2004) and HIV-AIDS patients (Adjei et al., 2003, 2004), food vendors (Ayeh-Kumi et al., 2009), drinking water (Kwakye-Nuako et al., 2007), vegetables and water bodies (Duedu et al., 2014). In their study, Squire et al. (2013) reported on the presence of *Cryptosporidium* oocysts in cattle from two agro-ecological zones of southern Ghana, thereby highlighting the importance of the parasite in farm animals. Accurate diagnoses of pathogens are

key elements for effective control strategies. *Cryptosporidium* oocysts are morphologically indistinguishable, and as such morphological identification alone may not be sufficient. Information on the molecular epidemiology of *Cryptosporidium* parasite in Ghana is scanty. The study reported in this paper was set out to identify *Cryptosporidium* parasites at the molecular level in herds of cattle that reside in three communities of southern Ghana.

Materials and Methods

Study areas

The study was carried out in Agona Nantifa, Osamkrom and Belekope. Agona Nantifa, a community located between latitudes 5°30'50"N and longitudes 0°35'55"W is in the Agona East District of the Central Region of Ghana. It has a coastal savannah vegetation and located along the Ayensu River. Osamkrom is located between latitude 5°29'15.0"N and longitude 0°41'09.8"W, in the Gomoa East District of the Central Region of Ghana has a similar vegetation type as Agona Nantifa. Belekope lies within latitudes 6°21'48"N and longitudes 0°6'12"W and is in the Lower Manya Krobo District of the Eastern Region of Ghana. It has a deciduous forest vegetation and located near the Volta River. These communities were selected because of the presence of river bodies, which are sources of treated water for surrounding communities. These communities have farm lands for grazing, hence the herds of cattle are managed on semi-intensive system. The cattle occasionally drink from the water bodies especially during the dry season, which may be source of oocysts transmission to humans.

Study design and sample collection

The study population was made up of cattle herds from the farms in these communities mentioned above. The farms were selected based on acceptance of the farmer/ owner to permit the use of their farm animals for the study. Willingness of the herder to assist with sample collection, such as restraining the animals was also one of the criteria for selection. Samples were collected in November/ December 2015, February/ March 2016 and June/ July 2016. These periods were about 2–4 months after the farm animals were dewormed by their owners. Prior to sample collection, questionnaire was administered to the farmers to assess farming practices, frequency of deworming and sources of drinking water. Each cattle herd was divided into three groups according to their age. These were pre-weaned (< 3 months old), 3 months to 1-year old, and > 1 year old. Fresh faecal samples were collected directly from the rectum of the animal. The samples were put into a suitable leak proof plastic container and labeled. They were placed on ice packs and transported to the parasitology laboratory of the Department of Animal Biology and Conservation Science, University of Ghana. The faecal samples were subsequently divided into two; one was stored in 10% formalin for morphological analysis (microscopy) and the other half stored in absolute ethanol for molecular analysis. Sample collection

was repeated the following day since the presence of *Cryptosporidium* oocysts are sporadically shed in faecal samples. All farm animals with diarrhoea were sampled for the study. The remaining animals were categorized into three groups as described above and samples collected from each group. The farm animals were marked with writing marker for the following day's repeat sampling. The samples were processed for morphological identification of *Cryptosporidium* oocysts and DNA extraction.

Morphological identification of *Cryptosporidium* oocysts

Faecal samples were processed using the formalin-ethyl acetate concentration method as previously described by Weber et al. (1992) using about two gram of the Formalin-fixed faecal sample placed in 15 μ l conical centrifuge tube. Slides were prepared by using 10 μ l aliquots of the resulting sediment. A drop of the sediment obtained from the formalin-ethyl acetate method was placed on a slide and allowed to dry. Differential staining was performed using the modified acid-fast staining technique. The slides were air-dried and scanned using the \times 400 magnification of the compound light microscope LEICA ICC50 HD and images captured on a computer for confirmation.

Genomic DNA extraction

All faecal samples were subjected to molecular examination. Prior to extracting genomic DNA, the samples were pre-treated in sodium hypochlorite solution to break open any oocyst to release its DNA. This was done by treating about 2 ml of ethanol-fixed faecal sample in 15 ml plastic tube with 5.25% sodium hypochlorite solution at 4°C for 10 minutes, and then washed five times in sterile water. Genomic DNA was extracted from the pre-treated sample by following the manufacturers recommended protocol of Zymo Research (ZR) Genomic DNATM Tissue Miniprep Kit.

Parasite DNA amplification

Nested PCR protocol was used in the amplification of the variable region of the coding gene for *Cryptosporidium* small-subunit (SSU) rRNA. The reaction mixture of each sample was made up to 25 μ l and comprised 12.5 μ l of 2 \times Taqmix (Buffer, MgCl₂, dNTPs, Taq polymerase), 0.8 μ l each of the 10 μ M primer pairs, 5.9 μ l of ddH₂O and 5 μ l of the template. Nested PCR was carried out to amplify the parasite DNA using the two sets of primers listed in Table 1. The SSUF1 and SSUR1 set of primer was used to amplify the gene after 35 cycles. Each cycle consisted of 94 °C for 45s, 55 °C for 45 s, and 72 °C for 1 min, with an initial hot start at 94 °C for 3 min and a final extension step at 72°C for 7 min. The SSUF2 and SSUR2 primer set was used for the secondary amplification using 2 μ l of the primary PCR product. A total of 35 cycles, each consisting of 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, with an initial hot start at 94 °C for 3 min and a final extension step at 72 °C for 7 min was carried out. The amplified and digested products were

fractionated on 2% agarose gel prepared with 1 × TAE buffer and visualized by ethidium bromide. The sizes of the PCR products were estimated by comparison with a 100 base pair molecular marker.

Ethical clearance

The Ethical Committee for the Basic and Applied Sciences (ECBAS) of the College of Basic and Applied Sciences of the University of Ghana, Legon approved the ethical clearance to conduct the study. Informed consent was sought from owners of the cattle ranches and arrangements made with the caretakers (herdsmen) for assistance before sample collection.

Results

Descriptive data

All five farmers interviewed mentioned that they deworm their farm animals every 4 months with oral 10% Abendazole. The cattle usually drink water from puddles, except during the dry season when they drink water from their respective water bodies in the communities.

Morphological identification of *Cryptosporidium* oocyst

A total of 242 individual cattle were sampled, of which 10% was diarrheic. No *Cryptosporidium* oocyst was morphologically identified by the compound light microscope in any of the samples (Table 2).

Molecular identification of *Cryptosporidium* DNA

Five faecal samples were positive for *Cryptosporidium* parasite DNA, representing prevalence of 2.07% (Table 3). All DNA positive samples came from pre-weaned calves from Farm D.

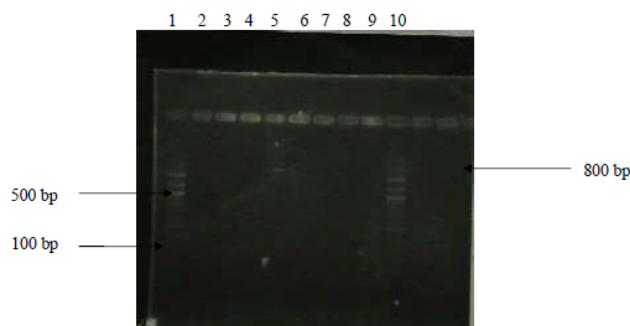


Figure 1. Photograph of ethidium bromide stained agarose gel electrophoresis of amplified PCR product. Lanes 1 and 10 are 100 base pair molecular weight markers. Lane 5 is a *Cryptosporidium* species positive sample amplified around 826–864 base pair. Lanes 2, 3, 4, 6, 7, 8, 9 are samples that were negative.

Discussion

In spite of the fact that it is zoonotic, cryptosporidiosis is one of the neglected tropical diseases that has received a relative little attention in Ghana. Studies on the disease in Ghana include that of Squire et al. (2013), who morphologically identified the *Cryptosporidium* oocysts in neonatal calves as an important causative agent of diarrhoea. Not all *Cryptosporidium* species are zoonotic as some are host specific, infecting only humans or vertebrate animals. A protozoan parasite responsible for this diarrhoeal disease, its transmission is seasonal. Even though sampling for our study was done in both dry and rainy seasons in Ghana, there was no *Cryptosporidium* oocyst morphologically identified by microscopy from the farm animals sampled. Oocysts survival is known to be optimal under cool damp conditions and ambient temperature (Davies et al., 2005; Jenkins et al., 2002; Lim et al., 1999; Olson et al., 1999). In their study, Fujino et al. (2002) observed that significant radiation from the sun and prolonged periods of drought result in rapid oocyst inactivation. Studies in Nigeria suggest the occurrence of *Cryptosporidium* infection in cattle herds where *C. bovis*, *C. ryanae* and *C. andersoni* were found (Ayinmode et al., 2010; Maikai et al., 2011). As almost all environmental survival studies have been conducted on oocysts of *C. parvum*, it is possible that oocysts from other species of *Cryptosporidium* are better able to tolerate dry and warm conditions.

Samples collected for the study were either diarrheic or non-diarrheic. This was done to increase the chances of obtaining the parasite oocysts. Diarrheic samples may sometimes result from other intestinal diseases such as worm infestation. Environmental factors such as rain or running water also influence the transmission of diarrhoeal diseases. No other endoparasite was identified by the direct smear technique. This may be attributed to the administration of Albendazole to all the farm animals sampled for the study, an exercise that is routinely done every four months. Squire et al. (2013) in their study did not mention the administration of dewormer by the farmers. This might have accounted for the wide variation in their observation and ours. The possibility of deworming drugs to wield anti-cryptosporidial effect could be investigated in future studies.

A study that compared three methods (modified Ziehl Neelsen technique, auramine phenol staining and a commercial enzyme immunoassay kit) of detecting *Cryptosporidium* oocysts in cattle faeces found that although the modified Ziehl Neelsen technique had a comparable or better sensitivity than the other methods, the specificity was lower (Brook et al., 2008). This method is inexpensive and the slides can be kept for permanent record. With the relatively large volume of faecal samples that cattle usually produce, microscopy sometimes is limited in diagnosing intestinal protozoan parasites such as *Cryptosporidium* except in heavily infected individuals. One advantage of the molecular technique is that even a trace amount of the parasite DNA could be recognized depending on the set of primer used. This is effective in cases of low

Table 1. Oligonucleotide primer pairs for amplification of the small subunit ribosomal RNA of *Cryptosporidium* species.

Primer pair	Sequence 5' → 3'	Expected amplified DNA band size
SSU F1	TTCTAGAGCTAATACATGCG	1,325 base pair
SSU-R1	CCCATTTTCCTTCGAAACAGGA	
SSU-F2	GGAAGGGTTGTATTTATTAGATAAAG	826-864 base pair
SSU-R2	AAGGAGTAAGGAACAACCTCCA	

Table 2. Age distribution of cattle examined for *Cryptosporidium* parasite

Farm designation	Farm location	Estimated cattle population	*Number of cattle examined	Age groups of cattle from which faecal samples were collected		
				Pre-weaned	3 months to 1 year old	>1 year old
A	Agona Nantifa	85	62	16	30	16
B	Agona Nantifa	47	22	6	10	6
C	Osamkrom	75	60	14	33	13
D	Belekope	70	52	12	18	22
E	Belekope	66	46	12	12	22
Total		343	242	60	103	79

(*)Total number of faecal sample is twice the number of cattle sampled since sampling was repeated using the same individual

Table 3. Diarrhoea status of cattle examined for *Cryptosporidium* parasite by Polymerase Chain Reaction (PCR)

Farm designation Farm location	Diarrheic cattle	Non-diarrheic cattle		Non-diarrheic cattle		Total number of cattle examined
		Number examined	Number positive	Number examined	Number positive	
A	Agona Nantifa	2	0	60	0	62
B	Agona Nantifa	0	0	22	0	22
C	Osamkrom	5	0	55	0	60
D	Belekope	9	5	43	0	52
E	Belekope	3	0	43	0	46
Total		19	5	223	0	242

(*)Number positive represents those that are *Cryptosporidium* positive by PCR

intensity of infection. The parasite species could also be determined and as such the zoonotic implications established.

In this study nested PCR protocol was used in the amplification of the variable region of the coding gene for *Cryptosporidium* small-subunit (SSU) rRNA. Five faecal samples were positive for *Cryptosporidium* parasite DNA, all of which were from pre-weaned calves from the same farm. Studies have shown that prevalence of cryptosporidiosis tends to increase with increasing herd size (Hamnes et al., 2006). Low herd size and lack of grazing also reduce the potential for farm-to-farm transmission, and can result in the fading out of disease (Anderson and May, 1992). Nonetheless, the relatively low prevalence recorded may be a true reflection of the case in these farms studied.

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