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Original Article

Molecular detection of *Cryptosporidium* species in street-sampled dog faeces in Ibadan, Nigeria

Ayinmode A.B.*, Obebe O.O., Falohun O.O.

Department of Veterinary Parasitology and Entomology, University of Ibadan, Ibadan, Nigeria

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ABSTRACT

Cryptosporidium, an intestinal protozoan parasite that infects a wide range of animals, is shed to the environment through the faeces of infected hosts that include humans and companion animals. Environmental contamination with infective oocysts increases the risk of transmission of this zoonotic pathogen to other susceptible host. In this study, *Cryptosporidium* was detected by polymerase chain reaction amplification of the 18S rRNA gene in 2.5% (5/203) of dog faecal samples randomly collected from 23 streets of Ibadan, southwestern Nigeria. Nucleotide sequencing of the 18S rRNA gene yielded two genotypes, *C. parvum* and *C. muris* in three and two samples, respectively. Further amplification of the gp60 gene-coding 60-kDa glycoprotein for subtype determination detected two isolates as *C. parvum* subtype IIc family. *C. parvum* subtype IIc is a human-derived pathogen while *C. muris* has been reported to infect rodents and even humans. The findings of this study suggest that free-ranging urban dogs could act as reservoirs and potential sources of environmental contamination with *Cryptosporidium* species of public health importance.

1. Introduction

Cryptosporidium is an apicomplexan protozoan parasite that causes chronic diarrhoea in humans (Xiao et al., 2004). The parasite is transmitted via ingestion of oocysts shed by infected host in faeces (Ryan et al., 2014). *Cryptosporidium* infection is usually subclinical in immunocompetent hosts, but may lead to a severe condition in children and immuno-depressed people (Current and Garcia, 1991; Chalmers and Davies, 2010; Bouzid et al., 2013).

Animals have been suggested to play some role in the transmission of the *Cryptosporidium* parasite to humans. Species of animal origin reported in humans include *C. meleagridis, C. felis, C. canis, C. cuniculus, C. ubiquitum, C. viatorum, C. muris, C. suis, C. fayeri, C. andersoni, C. bovis, C. scrofarum, C. tyzzeri, C. erinacei* and *Cryptosporidium* horse, skunk and the chipmunk I genotypes (Xiao and Fayer, 2008; Ryan et al., 2014). Abundant evidence exists to show the possibility of zoonotic transmission of this parasite between livestock (e.g. cattle) and humans (Fayer et al., 2000; Stantic-Pavlinic et al., 2003; Xiao and Fayer, 2008). However, the potential role of companion animals (dogs and cats) in *Cryptosporidium* zoonosis is still being studied (Xiao et al., 2007).

Dogs are very useful companions to humans all over the world and often maintain close contact with households and the environment (Morey, 2006). In most developed countries, dogs are kept indoors with proper care, unlike in developing nations where a lot of dogs still roam the streets. Faeces from parasite-infected dogs voided on the street have been shown to be potential sources of infection to humans and other animals (Zanzani et al., 2014; Ayinmode et al., 2016).

Reports on *Cryptosporidium* infection in dogs show that *C. canis* is the most detected species worldwide (Giangaspero et al., 2006; Xiao et al., 2007; Uehlinger et al., 2013). However, infection with other species like *C. parvum*, *C. meleagridis* and *C. muris* have also been reported in dogs (Hajdusek et al., 2004; Lupo et al., 2008; Yoshiuchi et al., 2010). Further, the possibility of transmission of host-adapted *C. canis* from dogs to humans has been suggested (Xiao et al., 2007).

In Nigeria, there are several reports of *Cryptosporidium* genotypes infecting humans (Akinbo et al., 2010; Ayinmode et al., 2012; Ayinmode et al., 2014), but there is no study that has determined the *Cryptosporidium* genotypes infecting dogs. Whereas there is evidence to show that dog faeces collected from public places in a Nigerian city contain zoonotic helminths that can infect humans (Ayinmode et al., 2016), there is no information on environmental contamination with zoonotic *Cryptosporidium* species in dog excrement shed on the streets in Nigeria. The present study was therefore designed to determine the genotypes of *Cryptosporidium* species shed in dog faeces on streets around human habitations in Ibadan, southwest Nigeria.

* Corresponding author.
E-mail address: ab.ayinmode@mail.ui.edu.ng (A.B. Ayinmode).

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2. Materials and methods

2.1. Study location and sampling

The study was conducted in Ibadan, Nigeria. Ibadan is one of the largest metropolitan cities in West Africa with coordinates 7° 23′ 47"N and 3° 55′ 0"E, an urban land area of 6800 km^2 (2600 sq. mi) and an estimated population of about 3.2 million (NPC, 2006). The mean total rainfall is 1420.06 mm with two peaks in June and September. Mean maximum and minimum temperatures are 26.46 °C and 21.42 °C, respectively while the relative humidity is 74.55% (Ajayi et al., 2012). Using a grid map of Ibadan as previously described (Offwell Woodland & Wildlife Trust (OWWT), 2016), dog faecal samples were collected at locations where packs of free-ranging urban dogs were seen on 23 randomly selected streets in Ibadan metropolis (Fig. 1).

A total of 203 faecal samples of free-ranging urban dogs were collected from these streets and preserved in 2.5% potassium dichromate at 4 °C until processed for molecular genotyping.

2.2. Cryptosporidium genotyping

DNA was extracted from the preserved faecal samples using the Ultra-pure® DNA Kit (Roche, Indianapolis, USA). *Cryptosporidium* species were detected by nested-polymerase chain reaction (n-PCR) amplification of a fragment (~590 bp) of the 18S rRNA gene using 18SiCF2 (forward: 5′-GACATATCATTCAAGTTTCTGACC-3′) and 18SiCR2 (reverse: 5′-CTGA AGGAGTAAGGAACAACC-3′), followed by a nested amplification using primers 18SiCF1 (forward: 5′-CCTATCAGC TTTAGACGGTAGG-3′) and 18SiCR1 (reverse: 5′-TCTAAGAATTTCACC TCTGACTG-3′) as previously described (Ryan et al., 2003). The 50 µl PCR reaction mixture contained 21.6 µl nuclease-free water (Roche, Indianapolis, USA), 25 µl master mix (Roche, Indianapolis, USA) containing pre-mixed Taq polymerase, $MgCl_2$ and dNTPs, 1.2 µl forward primer, 1.2 µl reverse primer and 1 µl DNA template. Both primary and secondary amplification were conducted at 94 °C for 5 min (initial denaturation), followed by 45 cycles of 94 °C for 30 s (denaturation), 58 °C for 30 s (annealing) and 72 °C for 30 s (extension), with a final extension of 72 °C for 10 min. All PCR amplicons were visualised by electrophoresis on 1.5% agarose gel after ethidium bromide staining. Amplicons that were positive on the gel were purified using the Roche Mini elute kit (Roche, Indianapolis, USA) and sequenced.

2.3. Cryptosporidium subtyping

Sub-genotyping was achieved by amplification of a 400-bp fragment of the 60-kDa glycoprotein (gp60) gene using primers AL3531 (5′-ATAGTCTCCGCTGTATTC-3′) and AL3533 (5′-G AGATATATCTTGG TGCG-3′) in the primary PCR, and AL3532 (5′-TCCGCTGT ATTCTCA GCC-3′) and LX0029 (5′-CGAACCACATTACAAATGAAGT-3′) in a nested step as previously described (Sulaiman et al., 2005). A 50 µl PCR reaction mixture was used as described for the 18S rRNA amplification except for the change in primers and DNA. *Cryptosporidium hominis* (TU502) and ultra-pure PCR water were used as the positive and negative controls, respectively. The secondary products were sequenced and compared with *Cryptosporidium* sequences found in the GenBank (http://www.ncbi.nlm.nih.gov/) using BLAST while sequence alignment was performed with Bioedit alignment editor (version 7.0.9.0). Phylogenetic trees were constructed using MEGA 5.2.2 program (www.megasoftware.net) to visualize the similarity between our sequences and selected reference sequences.

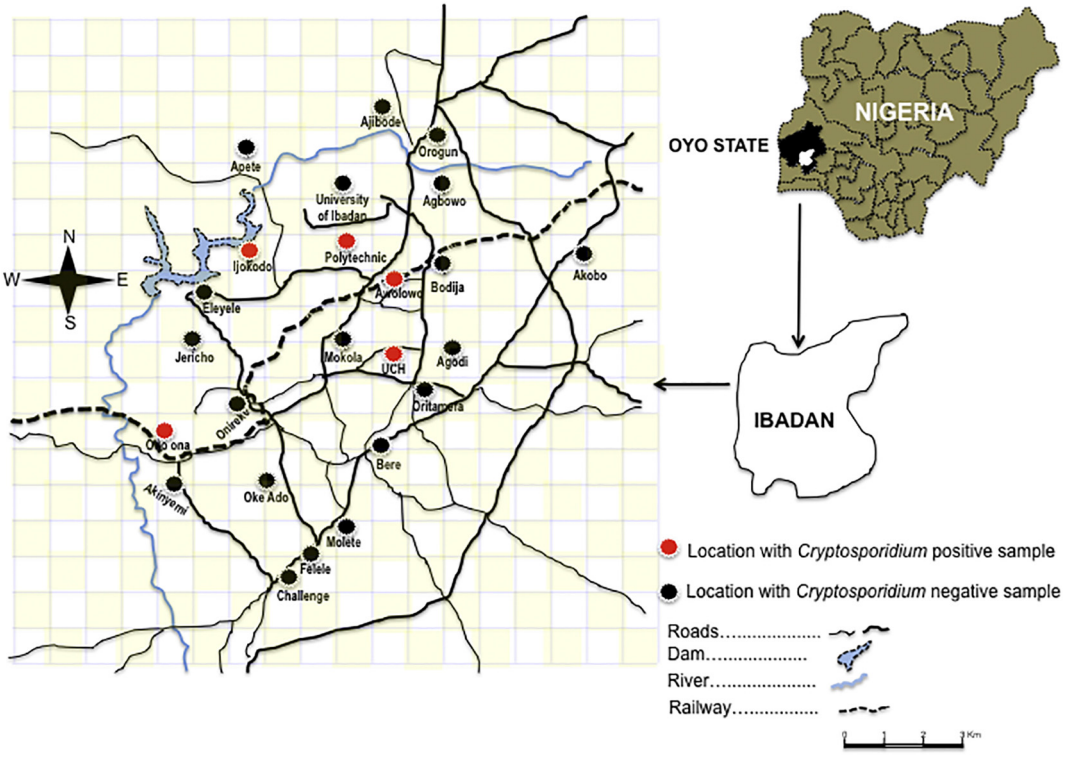

Fig. 1. Sampling locations and the distribution of *Cryptosporidium sp.*-positive faecal samples at different locations in Ibadan metropolis, Nigeria.