



## MOLECULAR DETECTION OF CRYPTOSPORIDIUM SPECIES IN DOMESTIC DUCKS SOLD FOR FOOD IN NIGERIAN LIVE BIRD MARKETS

Ayinmode, A. B., Falohun, O. O.

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

ayins2000@yahoo.com

### ABSTRACT

*Cryptosporidium* infections has been reported in several avian species including chickens, pigeons and game birds where these infections had been identified to cause either enteric or respiratory diseases. However, little data exists on the molecular characterization of *Cryptosporidium* species in ducks, especially those in frequent contact with humans. The aim of this study was to detect the *Cryptosporidium* species infecting domestic ducks in two major live bird markets. A total of 109 fresh faecal samples were collected from all the ducks available on sale in the two markets. The detection of *Cryptosporidium* species was conducted by microscopy. All positive samples were confirmed by the nested PCR amplification and the nucleotide sequencing of the 18S rRNA genes. The results demonstrated that the prevalence of *Cryptosporidium* infection in ducks using microscopy was 11.0 % (12/109). There was a higher prevalence 14.0 % (7/50) in ducks from Ibadan compared with those 8.5 % (5/59) obtained from Oyo town. All positive samples by microscopy were also positive using the nested PCR and

the DNA sequencing of the secondary PCR products from the 18S rRNA genes which revealed the presence of *Cryptosporidium parvum*. This study revealed that natural infections of *C. parvum* may occur in ducks in close contact with humans and other domestic animals and therefore suggests that cryptosporidiosis in ducks may be of public health importance.

**Key words:** *Cryptosporidium parvum*; ducks; faeces; markets; Nigeria

### INTRODUCTION

*Cryptosporidium* is a zoonotic protozoan parasite that causes chronic diarrhoea in domestic animals and humans [2, 3, 7, 25, 35]. Infection with the parasite can occur through the ingestion of infective oocysts and the disease has been known to be transmitted from animal-to humans and vice versa (humans to animals), and also from humans to humans [34, 35].

*Cryptosporidium* infections has been reported in sev-

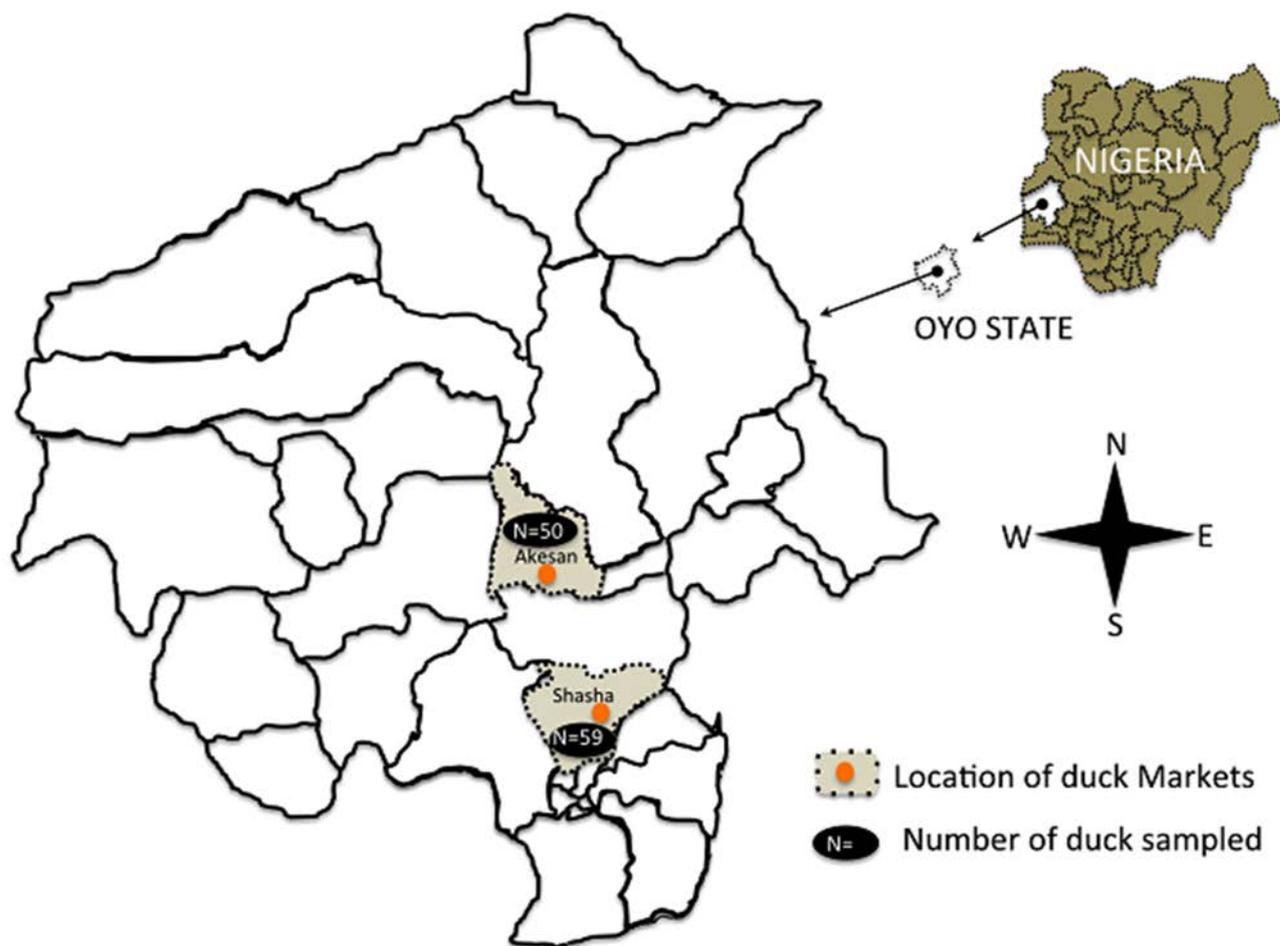


Fig. 1. The location of duck markets with the number of samples collected in Oyo State, Nigeria

eral avian species that includes domestic, pets, exotic, and wild birds, where it has been identified to cause either enteric or respiratory diseases [35]. There are three dominant *Cryptosporidium* species known to infect birds (*C. baileyi*, *C. galli* and *C. meleagridis*); other species reported are *Cryptosporidium* avian genotype I–V, goose genotype I–IV, Eurasian genotype and duck genotype [17, 20]. There are a few reports of *C. andersoni*, *C. muris*, *C. parvum*, and *C. hominis* infections in birds [15, 20, 21, 24, 36], although infection with these species/genotypes might have been accidental infections.

*Cryptosporidium baileyi* has been suggested to be the most common avian *Cryptosporidium* species because it infects many birds including ducks, domestic chickens, turkeys, geese, feral pigeon, lovebirds, budgerigars, cockatiels, quails and ostriches [8, 12, 30, 32]. There have been several reports of the detection of *C. meleagridis* in human samples and the pathogen is now suggested as zoonotic in nature [15, 18, 19, 29].

The increasing evidence of diversities of *Cryptosporidium* species in domestic and wild poultry that share the same ecology with human population underscores the importance of a need for continual world-wide surveillance of these birds for a better understanding of their possible role in the epidemiology of human cryptosporidiosis. This study was aimed at identifying the circulating species of *Cryptosporidium* in ducks in some live bird markets in Oyo state, Nigeria.

## MATERIALS AND METHODS

### Study location

This study was conducted in Akesan market, Oyo town and Shasha market in Ibadan located at 7°51'03.7"N 3°55'54.8"E and 7°29'00.2"N 3°54'37.4"E, respectively (Figure 1). Different breeds of ducks were maintained in local cages located on the ground and fed while the sellers waited for prospective buyers.

### Sample collection

A total of 109 fresh faecal samples were collected from all the ducks available in the two markets. The faecal samples were obtained from 50 local ducks (*Anas domestica*) and 59 Mallards (*Anas platyrhynchos*) in Shasha and Akesan markets, respectively. The samples were then stored at 4 °C until processed for molecular characterization.

### Detection of *Cryptosporidium* oocysts

The faecal specimens were concentrated by the formalin-ethyl acetate sedimentation method [14]. Briefly, 5 ml of the formalin-treated stool specimen was washed in 10 % formalin-saline, and the sediment, collected by centrifugation at 650 × 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 minutes and centrifuged at 500 × g for 5 minutes, 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 microscopic slide and dried for the acid-fast stain. *Cryptosporidium* oocysts were detected using a modified Ziehl-Neelsen staining method as described by Casemore [5]. The slides were observed under ×100 objectives for the presence of bright pink roundish oocysts. All positive samples were then processed for molecular genotyping.

### DNA extraction and genotyping

Faecal DNA was extracted using the Zymo research genomic DNA TM Tissue miniprep kit (Irvine, CA 92614, USA). *Cryptosporidium* species were detected by polymerase chain reaction (PCR) amplification of a ~590 bp fragment of the 18S rRNA gene using 18SiCF2 (5'-GACATATCATTC AAGTTTCTGACC-3') and 18SiCR2 (5'-CTGAAGGA GTAA GGAA CAACC-3') primers, followed by a nested amplification using primers 18SiCF1 (5'-CCTATCAGCTTTAGACGGTAGG-3') and 18SiCR1 (5'-TCTAAGAATTTCA CCTCT G A CTG-3') as previously described [27]. 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<sub>2</sub> and dNTPs, 1.2 µl forward primer, 1.2 µl reverse primer and a 1 µl DNA template. *Cryptosporidium hominis* (TU502) and ultra-pure PCR water were used as the positive and negative controls,

respectively. 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. Positive secondary PCR amplicons were sent for sequencing at Inqaba Biotec, Muckleneuk Pretoria, South Africa. The sequences were aligned using program MEGA 5 software ([www.megasoftware.net](http://www.megasoftware.net)). The sequences were compared with *Cryptosporidium* sequences found in GenBank (<http://www.ncbi.nlm.nih.gov/>) using BLAST. Phylogenetic trees to visualize the similarity between obtained nucleotide sequences and selected reference sequences was inferred using the neighbour joining method [28], with a model that best fit the alignment using Mega 6 [31]. The sequences obtained from the amplification of 18S rRNA secondary amplicons were deposited in the GenBank with the accession number MF400843, MF400845, MF400847, MF400848 and MF400849.

### RESULTS

Of the total 109 faecal specimens examined from ducks in this study, 12 (11.0 %) were positive for *Cryptosporidium* oocyst by microscopy. Ducks sold in the Shasha market, Ibadan showed a higher prevalence (14.0 %; 7/50) of *Cryptosporidium* infection than those sold in Akyesan market, Oyo (8.5 %; 5/59). The distribution among breeds of ducks revealed that 7 (14.0 %) of the Nigeria local breeds and 5 (8.5 %) of exotic breeds were positive for *Cryptosporidium* infections. The association between *Cryptosporidium* infection and age, breed and sex of ducks were not significant ( $P > 0.05$ ). Nested PCR amplification of the 18S rRNA gene showed that all the microscopy positive duck samples 12 (11.0 %) were positive for *Cryptosporidium* species. BLAST search analysis of the sequences obtained from the secondary PCR product identified the species as *Cryptosporidium parvum*. Phylogenetic analysis of the 18S rRNA sequences from 5 isolates from Oyo (2) and Shasha (3) had 100 % sequence similarity to *C. parvum* reference sequences (KY514066.1) isolated from pigeons in Columbia and they clustered together in the same clade of the Phylogenetic tree (Figure 2).

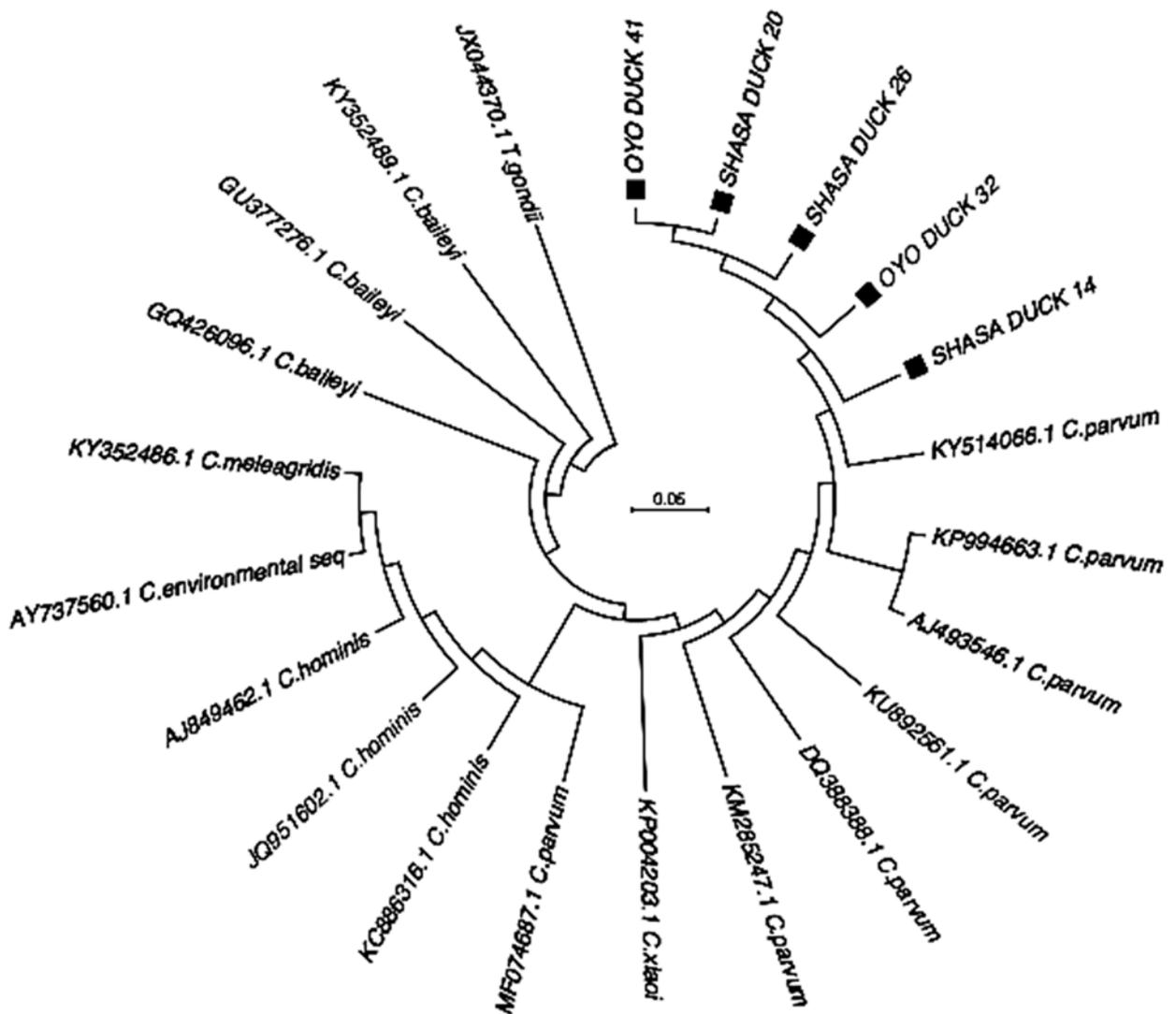


Fig. 2. Phylogenetic relationships inferred by neighbour-joining analysis of a fragment of the 18S rRNA gene sequence of *Cryptosporidium* from duck and reference sequences from the GenBank.

## DISCUSSION

Our study detected *Cryptosporidium* oocysts from ducks for sale in two different live bird markets in South-western Nigeria. A finding that suggested that these ducks are shedding *Cryptosporidium* oocysts in faecal droppings which contaminates the environment of the market areas. The overall prevalence of *Cryptosporidium* in ducks obtained in this study was 7.5 %. This was the same prevalence reported in Hungary from aquatic ducks [23] which was however lower than 16.3 % obtained in China from Pekin ducks [32], 49 % in wild ducks from USA [13] and 57 % in farmed ducks from Germany [26]. These differences may be attributed to the difference in the breeds of

birds, environmental factors, management practices as well as the immune status of the birds [4]. The low prevalence obtained in the present study may be attributed to the removal of the ducks from their natural environment into cages where they had limited access to infective *Cryptosporidium* oocysts. While there are several reports on *Cryptosporidium* infections in chicken, pigeon, game birds and Canada geese, there are only a few reports of the infections in domestic ducks and the present report to the best of our understanding is the first in Nigeria.

This study detected *C. parvum* as the genotype of the isolates detected by nested PCR and nucleotide sequencing of the 18S rRNA gene. This is a rare finding in domestic ducks as previous studies on *Cryptosporidium* infections

in ducks have often reported the occurrence of *C. baileyi*, *C. meleagridis* and *Cryptosporidium* duck genotype [1, 6, 11, 12, 19, 22, 32]. However, *C. parvum* infections have been demonstrated in experimental ducks [9]. Although the reasons for the occurrence of *C. parvum* in these ducks are not known, the present finding may be associated with the frequent contact with humans and other animals that may be sources of *C. parvum* infection in the markets. Ducks in the markets have contact with other animals like chickens, pigeons, guinea fowls and ruminants when the sellers bring them out of the cages for feeding. The findings of our present study demonstrate the occurrence of natural infection of *C. parvum* in ducks and suggest that domestic ducks may potentially serve as its potential reservoir and faeces from infected ducks sold in the sampled markets may be infectious to humans. Natural infection with *C. parvum* has also been reported in Canada geese (a species related to ducks) [10, 36]. *Cryptosporidium parvum* is one of the two most important pathogenic *Cryptosporidium* species infecting humans worldwide [16, 33].

In conclusion, although our study could not detect the subtype of the *C. parvum* due to the genetic marker used. The findings of this study show that natural infections of *C. parvum* may occur in ducks in close contact with humans and other domestic animals. It is therefore suggested that precaution should be taken by humans frequently in contact with ducks in markets and slaughter points to prevent the possibility of zoonotic infection.

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