

Original papers

Detection and molecular identification of *Cryptosporidium* species in laboratory rats (*Rattus norvegicus*) in Ibadan, Nigeria

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ABSTRACT. To study the occurrence of *Cryptosporidium* infection in laboratory rats (*Rattus norvegicus*) raised for experimental usage, 134 faecal samples were obtained from two rearing houses in Ibadan and examined for the presence of *Cryptosporidium* oocyst using the modified acid fast staining technique. *Cryptosporidium* species in 2 samples positive for microscopy were further characterized by a nested polymerase chain reaction (PCR) amplifying the 18S rRNA gene. Two of 134 samples were positive for the *Cryptosporidium* oocysts. Sequencing of the small-subunit rRNA amplicons identified the species in the two PCR positive samples as *Cryptosporidium andersoni* and *Cryptosporidium* rat genotype. These findings showed that laboratory rat is a potential reservoir for diverse *Cryptosporidium* species and suggests that laboratory rats should be screened for *Cryptosporidium* infection prior to experiments, especially where pathogen free animals are not available. This the first report to identify *Cryptosporidium* species infecting laboratory rats in Nigeria.

Key words: *Cryptosporidium* species, laboratory rat, microscopy, PCR, sequencing

Introduction

Cryptosporidium, a unicellular coccidian protozoan invading the gastrointestinal (GI) epithelium, is one of the most common aetiology of diarrhoea in livestock and humans worldwide [1]. Cryptosporidiosis can lead to persistent diarrhoea in infants and immunosuppressed hosts [2]. *Cryptosporidium* parasites are transmitted by the fecal-oral route via direct transmission and the ingestion of water or food contaminated with environmental resistant oocysts shed by humans (person to person) or by infected animals [1].

Cryptosporidiosis in rodents (wild, domestic and laboratory rats) has been investigated worldwide and the role of rodents as reservoir for species infecting humans has been documented [3]. There are also some available reports on *Cryptosporidium*

infection in *Rattus norvegicus* [4–10]. The *Cryptosporidium* genotypes and species commonly detected in rodents include rat genotype I-IV, skunk genotype, mouse genotype, chipmunk genotype and the species *C. ubiquitum*, *C. tyzzeri*, *C. muris*, *C. meleagridis*, *C. andersoni* and infrequently *C. parvum* [3,11–15]. Among these, *C. parvum*, *C. meleagridis*, *C. muris*, *C. andersoni* and *C. ubiquitum* are also known to infect humans [16].

This study was therefore aimed at investigating the occurrence of *Cryptosporidium* species and genotypes in the laboratory rats (*R. norvegicus*) in two rearing facilities in Ibadan, Nigeria.

Materials and Methods

Sample collection. Faecal samples were collected between October 2014 and May 2015

from 134 laboratory rats housed in two rearing facilities at the University of Ibadan, Nigeria. Fecal specimen were collected from individual rat cages and kept at 4°C until processed for microscopy and genotyping.

***Cryptosporidium* oocyst detection.** Faecal samples were examined for the presence of *Cryptosporidium* oocyst using fecal smear stained using the modified acid-fast method and microscopic examination. Slides were observed under a 100× objective 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 from acid-fast positive samples using the ultra-pure® DNA Kit (Roche, Indianapolis, USA). *Cryptosporidium* species were detected by a nested polymerase chain reaction (PCR) amplification of a ~590bp fragment of the 18S rRNA gene using 18SiCF2 (5'-GACATATCATT CAAGTTTCTGACC-3') and 18SiCR2 (5'-CTGA AGGAGTAAGGAACAACC-3') primers, followed by a nested amplification using primers 18SiCF1 (5'-CCTATCAGCTTTAGACGGTAGG-3') and 18SiCR1 (5'-TCTAAGAATTTACCTCTGACT G-3') as previously described [17]. The cycling conditions for both primary and secondary amplification were as follows: 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. *C. parvum* from mice (isolate

TU114) and ultra-pure PCR water were used as positive control and negative control, respectively. All PCR amplicons were visualised by electrophoresis in 1.5% agarose after ethidium bromide staining. Visible PCR amplicons were purified using Mini elute Kit (Roche, Indianapolis, USA) and sequenced at the Tuft University sequencing facilities (tucf.org). Sequence alignments were performed using program MEGA 5.2.2 (www.megasoftware.net) (ref.). Sequences were compared with *Cryptosporidium* sequences found in GenBank (<http://www.ncbi.nlm.nih.gov/>) using BLAST. Phylogenetic trees to visualise the similarity between obtained nucleotide sequences and selected reference sequences was inferred using the Neighbour-Joining method [18] with a model that best fit the alignment using Mega 6 [19].

Results

Microscopy detected *Cryptosporidium* oocyst in 1.5% (2/134) of rat fecal samples screened. Two positive samples detected by microscopy were confirmed as positive by nested PCR amplification of the 18S rRNA gene. BLAST search analysis of the sequences obtained from the secondary product identified the species as *Cryptosporidium andersoni* and *Cryptosporidium* rat genotype II. Phylogenetic analysis of the 18S sequences from the first isolate (UI8) identified as *C. andersoni*, had 97% sequence similarity with *C. andersoni* reference sequences (KT 922228.1) originating from calves and lambs in

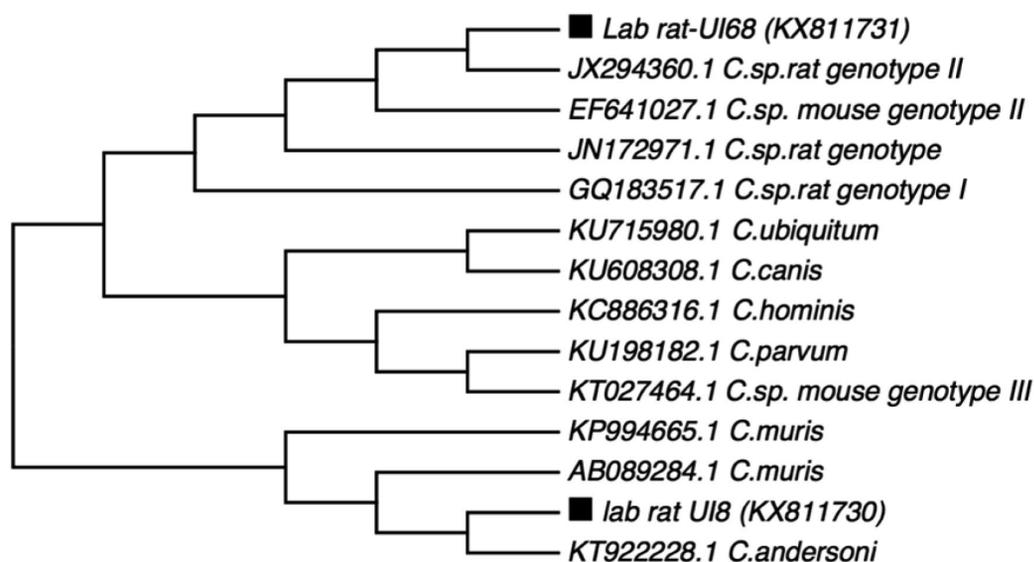


Fig. 1. Phylogenetic relationships inferred by neighbour-joining analysis of the SSU rRNA from *Cryptosporidium* from laboratory rat and reference sequences from the Genbank

Ethiopia [20] and clustered together in the same clade (Fig. 1). The second isolate (UI68) was found to be 99% similar to sequence JX294360.1 classified as *Cryptosporidium* rat genotype II from wild black rat (*Rattus rattus*) from Australia [21] and also clustered with it in a Neighbour-Joining phylogeny (Fig. 1). The nucleotide sequences obtained from the 2 isolates were deposited in the GenBank database under accession no. KX811730 (*C. andersoni*) and KX811731 (*Cryptosporidium* rat genotype II).

Discussion

The *Cryptosporidium* prevalence found in the present study is lower than the range of 5.0% to 39.0% reported in rodents from several continents [3,12,13]. It is, however comparable to 1.9% obtained in laboratory mice from China [14]. The disparity in results could be attributed to several reasons that could include differences in feeding, management system, geographical areas and also the sensitivity and specificity of the method of detection. Although, the source and route of infection for the laboratory rats in the present study are not known, it is however likely that the rats got infected by the ingestion of feed and water from sources contaminated with *Cryptosporidium* oocysts, or through the use of contaminated feeding or drinking troughs.

This study identified only two *Cryptosporidium* species (*C. andersoni* and *Cryptosporidium* rat genotype) after sequencing the DNA obtained from the amplification of the 18S gene. These species have been previously reported in various rodents worldwide [7,11–15,21,22].

C. andersoni is primarily a parasite of cattle [23], with few reports of its isolation from rodents [14] and birds [24]. Human infection by *C. andersoni* were also reported in Australia [25], China [26, 27], England [28], France [29], Iran [30] and Malawi [31]. Although, *C. andersoni* has been suggested to be a potential zoonotic species, but information about the infection source and transmission dynamics are yet to be elucidated [26].

Cryptosporidium rat genotype II was previously identified in laboratory and brown rats [14], snakes [32] and was also detected in water samples [33]. *Cryptosporidium* rat genotype II has been reported in wild rat and in some cases as a mixed infection with *Cryptosporidium* rat genotype III and IV [21]. It is however not yet known if these genotypes are

pathogenic to their host.

In conclusion, the nucleotide sequences and the phylogenetic analyses carried out on the limited number of isolates obtained in the present study show that laboratory rats can harbour diverse species of *Cryptosporidium* parasites. Further studies involving a larger population of rats from several rearing facilities are needed to determine the susceptibility of captive rats to different *Cryptosporidium* species and the possible public health implications of this infection. Furthermore, the present study also suggests that laboratory rodents that are not specific pathogen free should be screened prior to their use in experiments to avoid confounding effects.

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