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Short communication

The first finding of a natural infection of *Cryptosporidium muris* in a cat

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Abstract

Little is known about the species of *Cryptosporidium* infecting cats. The limited number of genetic studies conducted to date, have all identified *C. felis* as the species of *Cryptosporidium* in cats. We report a morphological and genetic description of a natural *C. muris* infection in a cat. Oocysts were viable and were successfully transmitted to laboratory mice. Further studies are required to determine the range and prevalence of *Cryptosporidium* species infecting cats.

Keywords: *Cryptosporidium muris*; Cat; Natural infection; Morphology; Genotyping

1. Introduction

Cryptosporidium is an enteric coccidian parasite that is an important cause of diarrhoeal illness in a variety of hosts including humans and domestic animals. Currently 15 species of *Cryptosporidium* are accepted as valid on the basis of biological and molecular chracterisation; *C. muris* which infects rodents, *C. andersoni* which infects cattle; *C. parvum* which infects cattle, humans and other mammals; *C. bovis* in cattle and sheep; *C. suis* in pigs; *C. hominis* which infects humans; *C. meleagridis*, *C. baileyi* and *C. galli* in birds; *C. serpentis* and *C. saurophilum* in snakes and lizards; *C. molnari* in fish, *C. wrairi* from guinea pigs, *C. felis* in cats and *C. canis* in dogs (cf. Xiao and Ryan, 2004; Fayer et al., 2005).

C. felis in cats was first described in 1979 (Iseki, 1979) and genetically characterised in 1998 (Sargent et al., 1998). Little is known however, about the prevalence and distribution of *Cryptosporidium* species infecting cats. Epidemiological surveys conducted in Australia, Japan, Germany, Italy, the United States and the United Kingdom have identified *Cryptosporidium* oocysts in 0.75–12.2% of cats (cf. Fayer et al., 1997; Morgan et al., 1998; Hill et al., 2000; Spain et al., 2001; McGlade et al., 2003; Cirak and Bauer, 2004; Epe et al., 2004). Only a handful of genetic studies have been conducted on feline-derived *Cryptosporidium* isolates and all have identified *C. felis* as the species infecting cats (Morgan et al., 1998; Gasser et al., 2001; Hajdusek et al., 2004; Ryan et al., 2003). In the present study, we report biological and genetic characterisation of the first finding of a natural *C. muris* infection in a cat.

2. Materials and methods

In December 2004, fecal specimens from a 3.5-month-old British short-hair cat with gastroenteritis, were sent to the State Veterinary Institute, Prague, for parasitological examination. Microscopic examination was conducted using a standard concentration,

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flotation–centrifugation method (Breza, 1957; Pavlasek, 1991). Between 13 December 2004 and 14 January 2005, the owner submitted an additional six fecal specimens for analysis, all of which were positive for *Cryptosporidium* with the exception of the final sample submitted on 14 January.

Oocysts were purified from fecal samples and approximately 10^5 inoculated perorally into five 35-day-old mice that were negative for *Cryptosporidium* by microscopy prior to the start of the experiment. A control group of mice were infected with *C. muris* oocysts obtained from a naturally infected wild sewer rat. An uninfected negative control group were also included. Fecal samples from the mice were daily examined for the presence of oocysts from days 5 to 15 PI, and until day 70 PI in 2 up to 4-day intervals. The control group of mice was monitored in 4-day intervals.

DNA was extracted from oocysts from a stool sample from the cat, and from oocysts obtained via passage in mice using a Qiagen Stool Kit (Qiagen, Hilden Germany). A two-step nested PCR protocol was used to amplify the 18S rDNA gene as previously described (Ryan et al., 2003). *C. serpentis* DNA was used as a positive control. PCR products were purified using Qiagen spin columns (Qiagen, Hilden, Germany) and sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions except that the annealing temperature was raised to 60 °C. Sequences were analysed using SeqEd v1.0.3 (Applied Biosystems, Foster City, CA).

3. Results

Microscopic examination revealed the presence of *Toxocara cati* eggs and oocysts, which resembled *C. muris* in size and shape (8.0 μm × 4.8 μm, mean width/length index 0.6) in all samples (see Fig. 1). Infectivity studies in mice revealed that oocysts from the feline-derived isolate were observed on day 11 PI, with the patent period lasting 42 days. The control mice infected with rodent *C. muris* isolate started to shed oocysts on day 10 PI, and continued to shed for 68 days. In both isolates, the maximum intensity of oocyst shedding was from days 20 to 35 PI. Negative control mice, which received no oocysts remained negative for *Cryptosporidium* throughout the experiment.

Partial sequence analysis of the 18S rRNA gene obtained from the feline-derived and mouse passaged isolates identified the species as *C. muris* (100% homology) (see Fig. 2). The identity of the isolates was also confirmed using BLASTn searches of GenBank.

4. Discussion

C. muris infects rodents and natural infection have also been reported in a wide range of additional hosts including hamster, squirrels, Siberian chipmunks, wood mice (*Apodemus sylvaticus*), bank voles (*Clethrionomys glareolus*), rock hyrax (*Dolichotis patagonum*), bactraian camels, mountain goats, humans and cynomolgus monkeys (cf. Xiao et al., 2004). In addition, experimental *C. muris* infections have been reported in dogs, rabbits, lambs and cats (Iseki et al., 1989).

In the present study, morphological and genetic characterisation of a feline-derived *Cryptosporidium* isolate has confirmed the identity of the species as *C. muris*. The feline-derived *C. muris* oocysts were viable and infectious as they were successfully transmitted to laboratory mice. This is the first report of a natural *C. muris* infection in a cat. Until now, the only species thought to naturally infect cats was *C. felis*.

The cat was purchased by the owner in a local pet shop. Seven days after purchasing the cat, it presented with gastroenteritis and vomiting and was treated with Streptonamid, Cerucal, No-Spa and Farmatan plv

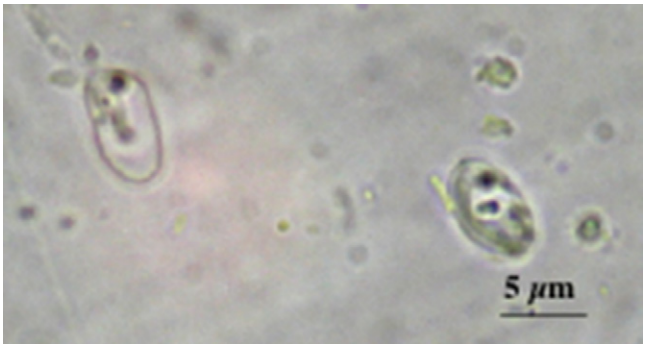

Fig. 1. *Cryptosporidium muris*-like oocysts from a British short-hair cat at 1000× magnification.