

CASE STUDY

## Possible zoonotic transmission of *Cryptosporidium felis* in a household

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In humans, the risk of contracting cryptosporidiosis caused by *Cryptosporidium felis* is considered to be relatively low, and most of the confirmed cases have been observed in immunocompromised patients. Both anthroponotic and zoonotic transmission routes have been suggested. Here, we report a case of suspected zoonotic transmission of *C. felis* from a cat to a human. The cat developed diarrhea several months before such symptoms were displayed by its owner, a 37-year-old immunocompetent woman. The presence of identical *C. felis* SSU rRNA, HSP70, and COWP gene sequences was verified in both hosts. In conclusion, it is highly probable that the cat was the initial source of infection and not the opposite. Our results show that *Cryptosporidium* infection can be transmitted from pets to humans and that molecular analysis is needed to confirm the identity of the oocysts.

Keywords: *Cryptosporidium felis*; cryptosporidiosis; molecular characterization; zoonotic transmission

Responsible Editor: Josef Järhult, Uppsala University, Sweden.

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Received: 7 May 2015; Revised: 26 August 2015; Accepted: 9 September 2015; Published: 6 October 2015

Most cases of human cryptosporidiosis are caused by either *Cryptosporidium hominis* or *Cryptosporidium parvum*, the latter of which also infects a number of other mammals, mainly calves and lambs, and is thus responsible for most zoonotic infections in humans. The use of molecular methods has revealed that humans can be infected with several other zoonotic *Cryptosporidium* species as well, including *Cryptosporidium meleagridis*, *Cryptosporidium cuniculus*, *Cryptosporidium ubiquitum*, *Cryptosporidium canis*, and *Cryptosporidium felis* (1–3), although recent findings suggest that some of these infections arise through anthroponotic rather than zoonotic transmission (1, 4). Hence, the role of companion animals such as dogs and cats in transmission of human cryptosporidiosis is not clear. Here, we investigated a case of suspected zoonotic transmission of *C. felis* between a cat and a human in Sweden.

### Materials and methods

The present investigation was part of a national project assessing *Cryptosporidium* species and subtypes in sporadic and outbreak-associated cryptosporidiosis cases from January 2013 to December 2014 (manuscript in preparation).

In this study, a cat and its owner were subjected to extensive clinical and epidemiological evaluation due to the suspicion of zoonotic transmission of *C. felis*. Approval was obtained from the Ethical Review Board at Karolinska Institutet, Stockholm, Sweden.

### Laboratory investigations

Fecal specimens for microscopy and molecular investigation were collected as indicated in Fig. 1. The presence of *Cryptosporidium* oocysts in the specimens was investigated by microscopy carried out after modified Ziehl–Neelsen staining (mZN) and/or staining with an anti-*Cryptosporidium* FITC-conjugated monoclonal antibody (Mab) (Cellabs Pty Ltd., Brookvale, NSW, Australia). DNA was extracted directly from the human stool specimen using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Before extraction, oocysts were disrupted using a Mini-Bead Beater (Biospec Products Inc., Bartlesville, OK, US). For the feline specimens, oocysts were isolated on a sucrose gradient prior to oocyst disruption, and DNA was extracted as described above.

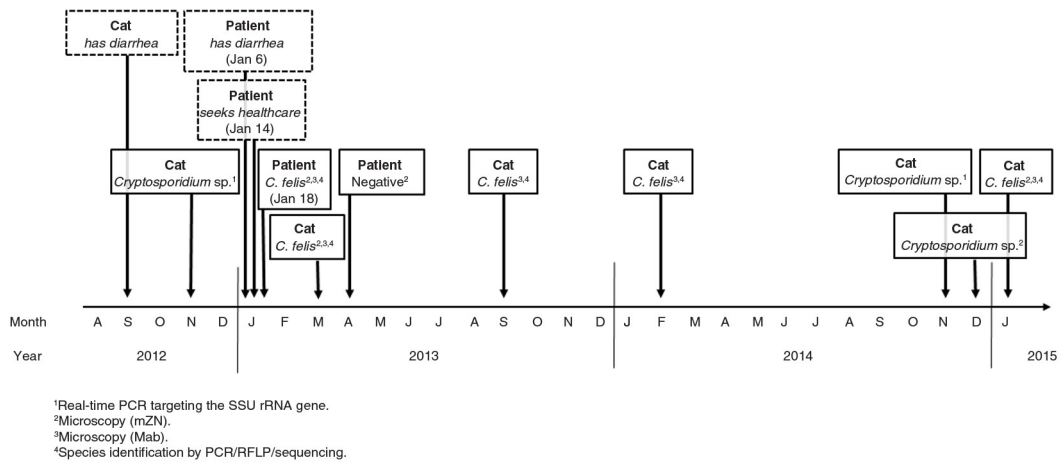


Fig. 1. Investigation of *C. felis* transmission.

The *Cryptosporidium* oocyst wall protein (COWP) and small subunit rRNA (SSU rRNA) genes were investigated by PCR and restriction fragment length polymorphism (RFLP) analysis, followed by sequencing of the obtained amplicons (5, 6). PCR and sequencing of the 70-kDa heat shock protein (HSP70) gene were also carried out (7). To ensure coverage of the full length of the amplified HSP70 product (approx. 1,900 bp), two additional forward sequencing primers were designed for *C. felis* based on GenBank accession number AF221538: *C.felis*SF1 5'-TGAGGACTTTGACAACAGGC-3' and *C.felis*SF2 5'-CTCCCAGAGGAGTGCCACAG-3'.

Four DNA samples previously extracted from stool specimens from humans with confirmed *C. felis* infection (8, 9) were included for comparison and analyzed at the HSP70 locus. To obtain *C. felis* DNA from feline hosts for a similar comparison, *Cryptosporidium* SSU rRNA PCR followed by RFLP was performed in 27 DNA samples originating from an earlier investigation of feline fecal specimens positive for *Giardia* (not previously investigated for *Cryptosporidium*) (10). The *C. felis*-positive samples obtained in this matter were further characterized at the HSP70 locus as outlined above.

Sequences were edited and analyzed using the BioEdit Sequence Alignment Editor version 7.0.9.0. The sequences were compared with sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST; NCBI www.ncbi.nlm.nih.gov/blast/Blast.cgi).

**Nucleotide sequence accession numbers**

Representative nucleotide sequences have been deposited in GenBank under accession numbers, KP642067–KP642069.

**Results**

**Cat history**

The cat described here is a castrated male Maine coon. At the age of 12 weeks (Fig. 2), it was moved to a new

environment where there were several other cats. However, it was returned to the original owner 7 weeks later, at which time it had watery diarrhea (Fig. 1). Local veterinarians examined the cat on several occasions, and repeated fecal sampling was performed to rule out common parasite infections. No improvement in the animal's condition was achieved by treatment with metronidazole, dihydrostreptomycin, prednisolone, or several different diets and therefore a fecal sample was sent to Idexx Laboratories in Germany for analysis of diarrhea-causing agents, including *Cryptosporidium*. At the age of 5 months, the cat was underweight and had a dull coat and chronic diarrhea (Fig. 2), and accordingly, it was taken to a specialized animal hospital. The laboratory results from Germany indicated that the cat was infected with *Cryptosporidium* but showed no other diarrhea-related agents. The cat's condition improved after treatment with the veterinary macrolide antibiotic tylosin, which has a bacteriostatic effect. At that time, the woman who owned the cat told the veterinarian that she herself was experiencing intestinal problems and the veterinarian recommended that she visit a health center for *Cryptosporidium* investigation.

**Follow-up of the cat**

The health and parasitological status of the cat was further examined on several occasions after the initial investigation (Fig. 1). At the age of 1 year (Fig. 2), the animal was asymptomatic but still positive for *C. felis* by microscopy and PCR and since then it has been essentially healthy, albeit with several periods of diarrhea. Samples subsequently taken on four additional occasions were all positive for *Cryptosporidium* (Fig. 1). During this period, the cat was treated twice with tylosin and once with azithromycin, followed by a multi-strain probiotic. At the time of writing, the cat was still under treatment with probiotics and had predominantly been clinically stable since this treatment was initiated.



Fig. 2. The cat at different ages: (a) 12 weeks old, healthy; (b) 5 months old, with chronic diarrhea; (c) 1 year old, healthy.

### Patient history

The cat owner is a 37-year-old woman. Prior to the *Cryptosporidium* infection, she had been diagnosed with irritable bowel syndrome (IBS), lactose intolerance, vitamin B12 deficiency, and asthma. She used inhaled steroids but no proton-pump inhibitors. The *Cryptosporidium* infection presented as watery, non-bloody diarrhea (Fig. 1), with 15–20 bowel movements a day, fever (38°C), and arthralgia in the legs. The woman was treated with oral rehydration but was not hospitalized. Her condition gradually improved, and she recovered in 10 days. Initial fecal microscopy identified *Cryptosporidium* oocysts, whereas follow-up microscopy 2 months later was negative (Fig. 1). The woman was very meticulous about good hand hygiene after cleaning the cat's litter box, but she allowed the cat to sleep in her bed. No other members of the household developed diarrhea.

### Laboratory results

The results of microscopy and PCR analysis of patient and feline specimens are shown in Fig. 1. Molecular characterization of the isolates from the patient and the cat revealed matching SSU rRNA gene sequences with 100% identity to the reference *C. felis* sequence (GenBank acc. no. AF108862). The COWP sequences obtained also showed 100% identity to each other and to the reference sequence (GenBank acc. no. AF266263). Likewise, the HSP70 sequences were identical to each other and showed 100% identity to the reference sequence (GenBank acc. no. AF221538). Considering the 27 feline fecal DNA samples, the PCR-RFLP analysis of the SSU rRNA locus identified five specimens (18.5%) positive for *C. felis*. PCR and

subsequent sequencing at the HSP70 locus of these five feline isolates and the four additional isolates of humans revealed that all the feline isolates and two of the patient isolates were 100% identical to the case isolates. However, the two remaining isolates from humans differed by a single nucleotide (G to T) at position 197 from the start of the reference sequence, resulting in an amino acid change of glutamine to histidine. All isolates were identical at the repetitive region of the 3' end of the HSP70 gene.

### Discussion

To the best of our knowledge, we are the first to report the use of molecular methods to confirm a case of cryptosporidiosis caused by transmission of *C. felis* from a cat to a human. The plausibility of *Cryptosporidium* being passed from cats to humans has been suggested in a few earlier studies. For example, in 1983 Koch et al. (11) described cryptosporidiosis in an immunocompromised patient whose cat also harbored *Cryptosporidium* oocysts. Similarly, Egger et al. (12) reported a case in which an 8-year-old boy contracted cryptosporidiosis during a visit to a farm; *Cryptosporidium* oocysts were found in a cat but not in the investigated calves on the farm, and thus the authors suggested that the cat was the source of infection. However, neither of these cases was confirmed by molecular methods.

Another case of unusual zoonotic *Cryptosporidium* transmission has been described from Peru (13). In the cited study, the SSU rRNA gene sequences of the parasites obtained from a dog and children living in the same household were identical to each other and to the reference *C. canis* sequence, but it was not possible to rule out that

the dog had been infected with *C. canis* from the children. Recently, evidence of zoonotic transmission of *C. meleagridis* from chickens to a human was also acquired by sequence analysis of the SSU-rRNA and HSP70 loci (14), and this conclusion was further strengthened by the findings of identical 60 kDa glycoprotein (GP60) subtypes in specimens from the birds and the patient (15).

In the present study, the HSP70 sequences from the cat and the patient were 100% identical to each other (1,885/1,885 bp), but identical sequences were also found in most of the other investigated *C. felis* isolates from cat and humans. Only two of the human isolates showed a minor discrepancy compared to the rest of the isolates. The highly polymorphic repetitive part of the 3' end of the HSP70 gene, which has been successfully used for subtyping of *C. hominis* and *C. meleagridis* (14, 16, 17), did not vary between the different *C. felis* isolates examined in the current study. The SSU rRNA gene PCR products of *C. felis* are difficult to sequence, because different copies of the gene vary in length (18). Indeed, in our study it was necessary to perform repeated PCR and sequence analyses of this gene to achieve acceptable results (data not shown). Together, these observations underline the lack of high-resolution subtyping methods for *C. felis*. The highly polymorphic GP60 gene represents the locus used most often to subtype *Cryptosporidium* species, and to date it has been identified in 16 different species/genotypes (2, 19, 20), although it has not yet been characterized in *C. felis*. Hopefully, whole-genome sequencing of this species will enable typing methods with high resolution in the near future.

Prevalence of *Cryptosporidium* in cats varies considerably, ranging from 0 to 29.4% depending on the population investigated and the evaluation methods used (21, 22). In studies employing species identification, most cases of feline cryptosporidiosis have been attributed to *C. felis*, and detection of *C. parvum* or *C. muris* has only occasionally been reported (23–25). In Sweden, the prevalence of *Cryptosporidium* infection in cats is not known, but the present observation that 18.5% (5/27) of fecal specimens from diarrheic cats with giardiasis were positive for *C. felis* suggests that occurrence of feline cryptosporidiosis is underestimated in this country. However, inasmuch as *Cryptosporidium*–*Giardia* co-infection seems to be rather common in cats, the observed rates must be interpreted with caution (22, 26, 27).

Human *C. felis* infections have been reported in most parts of the world, often with more frequent findings in developing countries than in industrialized nations (21, 28). Studies in Europe have identified varying numbers of *C. felis* cases (9, 29, 30): 0.26% of investigated samples (38/14,469) positive for *Cryptosporidium* in England and Wales, 1.0% (2/193) in Sweden, and 4.5% (15/310) in France. Elwin et al. (30) recognized immunosuppression as a risk factor for *C. felis* infection, thus evaluation of different

study populations is probably the main explanation for the observed discrepancies.

The cat owner in our study had a number of previous diagnoses, including IBS, although none of them were considered to facilitate *Cryptosporidium* infection, and her medications included inhaled but not systemic corticosteroids. Moreover, she was very meticulous about her hand hygiene after cleaning the cat's litter box. Nevertheless, the infectious dose of *Cryptosporidium* is very low, and hence it is highly likely that the mode of transmission in this case can be explained by the fact that the cat used to sleep in its owner's bed.

The cat's health status has been monitored since the first incidence of *Cryptosporidium*, which has shown that the animal was still excreting oocysts after 2 years of follow-up. The reason for this remains unclear. The cat has been investigated continuously for other infections and underlying diseases, but nothing noteworthy has been found that can explain why the oocyst excretion has continued. The cat's general condition is good, and currently the main cause of concern is the potential risk of reinfection of the owner or infection of other people or cats in the household, but, fortunately, no such events have yet occurred. FitzGerald et al. (23) have described a case in which a cat that was originally infected with two *Cryptosporidium* species (*C. muris* and *C. felis*) was still excreting oocysts 1 year later, but it appears that very few studies have included long-term follow-up of cats infected with *Cryptosporidium*.

Considering that the cat in our study developed symptoms of cryptosporidiosis before its owner did, and that human cryptosporidiosis caused by *C. felis* is rare in Sweden, we conclude that it is highly probable that the cat was the initial source of infection, and not the opposite. Our results also indicate that, as soon as zoonotic *Cryptosporidium* infection is suspected, molecular analysis should be performed to confirm the identity of the oocysts.

## Acknowledgements

The authors are grateful to the patient/cat owner for making this investigation possible and also for contributing the photographs. This work was part of a project entitled 'Integrated Risk Characterization and Risk Handling for *Cryptosporidium*' funded by a grant from the Swedish Civil Contingencies Agency.

## Conflict of interest and funding

The authors declare that they have no conflicts of interest.

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