



## Identification of *Cryptosporidium felis* in cats from a shelter: A Case Report

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**Abstract:** *Cryptosporidium felis* is an intestinal protozoan parasite of clinical and zoonotic importance, commonly associated with gastrointestinal disease in cats, particularly under conditions of poor hygiene and insufficient preventive care. The present case report describes the identification of *C. felis* infection in four domestic cats originating from the same outdoor shelter in Timișoara, Romania, all belonging to a single owner. The animals, aged 8 months, 1 year, 2 years, and 3 years, were presented for veterinary evaluation due to persistent diarrhea and behavioral changes. None of the cats had been vaccinated or dewormed, and they were maintained in a moderately hygienic environment. Diagnostic investigations were performed to determine the underlying etiology. Initial screening using a rapid antigen test suggested *Cryptosporidium* infection. Genomic DNA was extracted from fecal samples before molecular analysis. The 18S rRNA gene of *Cryptosporidium* was subsequently amplified for sequence and phylogenetic analyses. Based on sequence analysis, the detected oocysts were identified as *Cryptosporidium felis*. Comparative analysis revealed high similarity with isolates previously reported from human and water sources, highlighting the zoonotic potential of this parasite. These findings emphasize the importance of including cryptosporidiosis in the differential diagnosis of chronic or treatment-resistant diarrhea in cats, particularly in shelter environments. Furthermore, the study underlines the value of combined diagnostic approaches and the need for improved hygiene and preventive veterinary care to reduce transmission risk and potential public health implications.

### • Introduction

*Cryptosporidium* spp. are ubiquitous protozoan parasites of the phylum Apicomplexa, recognized as important causes of gastrointestinal disease in humans and a wide range of domestic and wild animals. Companion animals, particularly dogs and cats, play an important role in the epidemiology of cryptosporidiosis due to their close contact with humans, while cats are recognized as potential reservoir hosts. Shelter environments represent high-risk settings for the transmission of intestinal protozoa due to factors such as high animal density, stress, and variable hygiene conditions. Advances in molecular epidemiology, including PCR amplification and sequencing of genetic markers allow accurate identification of *Cryptosporidium* species and evaluation of their zoonotic potential. The present study aims to contribute to this field by characterizing *Cryptosporidium felis* infection in cats, highlighting its clinical relevance and potential implications for both veterinary and public health.

### • Material and method

The present study describes a clinical case involving four domestic cats presented to the University Veterinary Clinics of Timișoara, Romania. The animals had been rescued from the street and housed together in a rescue environment, allowing close contact and potential pathogen transmission. All cats showed similar clinical signs, including apathy and persistent diarrhea, and none had a history of vaccination or antiparasitic treatment. Fecal samples were collected for parasitological and molecular investigations. Initial screening for *Cryptosporidium* infection was performed using a rapid antigen detection test, and all animals tested positive for *Cryptosporidium* spp. infection. To confirm the infection, conventional nested polymerase chain reaction (PCR) targeting the small subunit ribosomal RNA (SSU rRNA) gene was performed following DNA extraction from fecal samples. The primary PCR targeted an approximately 1325 bp fragment of the SSU rRNA gene using the primers SSU-F2 (5'-TTCTAGAGCTAATACATGCG-3') and SSU-R2 (5'-CCCATTTCCTTCGAAACAGGA-3'). The secondary PCR amplified an approximately 850 bp fragment using the primers SSU-F3 (5'-GGAAGGGTTGTATTTATTAGATAAAG-3') and SSU-R4 (5'-CTCATAAGGTGCTGAAGGAGTA-3'). PCR products were analyzed by horizontal electrophoresis in a 1.5% agarose gel, and the amplified products were subsequently subjected to sequencing. The obtained sequences were compared with reference sequences available in the GenBank database using BLAST analysis.

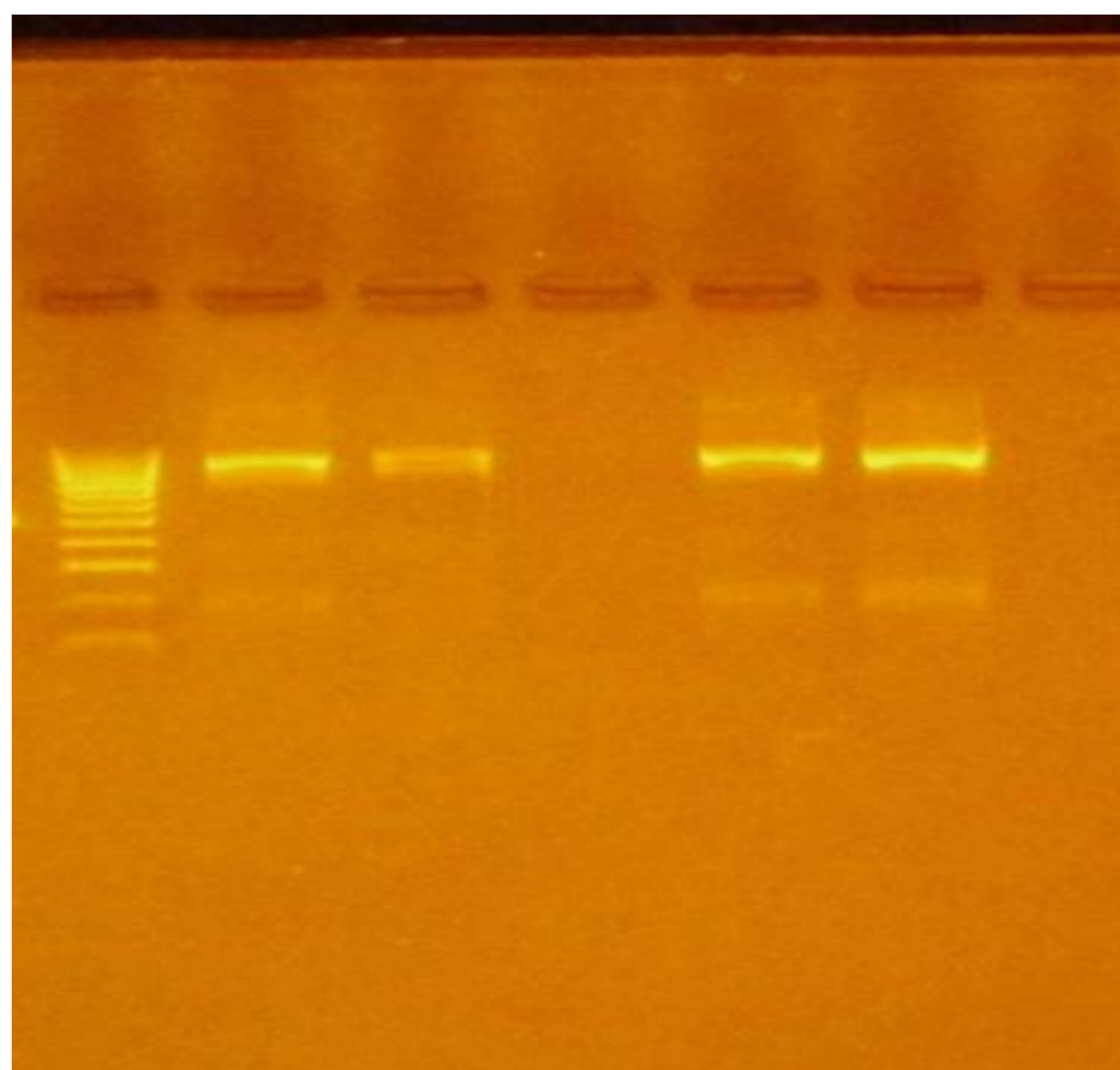
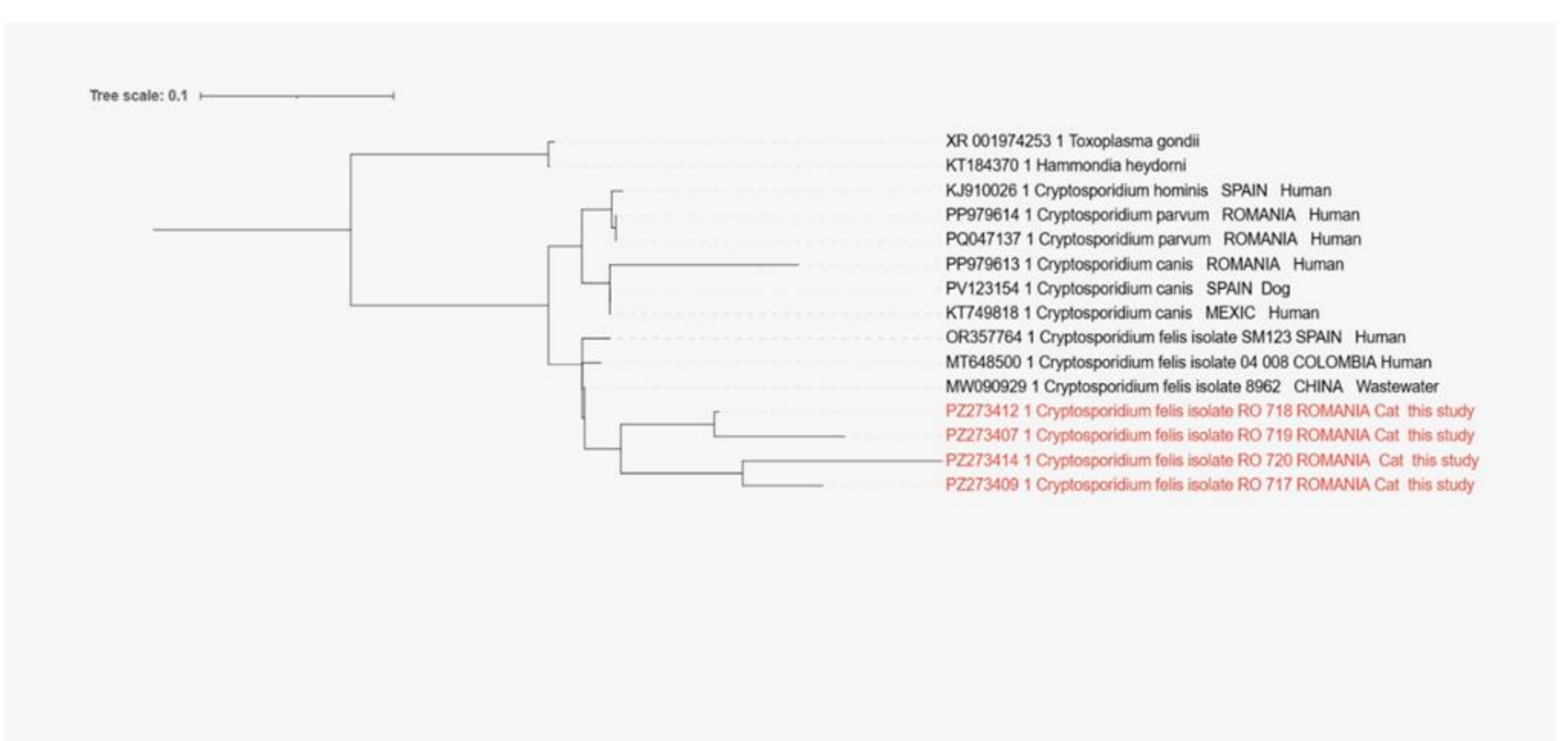


Image of the 1.5% agarose gel electrophoresis of the positive analyzed samples.

### • Results and discussions

All four fecal samples tested positive for *Cryptosporidium* spp. using the rapid antigen detection assay. Molecular analysis by nested PCR successfully amplified the SSU rRNA gene in all samples, generating products of the expected size (~850 bp), confirming the presence of *Cryptosporidium* DNA. Sequencing of the PCR products enabled molecular characterization of the isolates, and BLAST analysis showed that all sequences were most closely related to *Cryptosporidium felis*. Some isolates showed similarity with *C. felis* isolate (MT648500.1) previously identified in human-derived samples from Colombia. Phylogenetic analysis demonstrated that all four isolates clustered within the *Cryptosporidium felis* clade, suggesting a common source of infection or transmission within the same environment. The obtained sequences were deposited in the GenBank database under accession numbers PZ273407, PZ273409, PZ273412, and PZ273414.

In cats, *Cryptosporidium* infections are often reported as subclinical, but clinical signs such as diarrhea can occur, especially in young or stressed animals. Similar findings were reported by Tzannes et al. (2007), who associated enteric protozoa with gastrointestinal disease in cats. All isolates were identified as *Cryptosporidium felis*, consistent with studies by Li et al. (2021) and Oliveira et al. (2021), which showed that *C. felis* is the main species infecting cats, both in household and stray populations. Multi-cat environments are known to favor the transmission of intestinal parasites due to close contact, shared resources, and increased environmental contamination, as previously described in shelter-based studies by Enemark et al. (2020). The phylogenetic analysis supports this idea, as all isolates grouped closely together and clustered within the *C. felis* clade, suggesting local transmission within the same environment. Although *C. felis* is generally considered less important from a zoonotic perspective, reports of human infections highlight its potential public health relevance.



Phylogenetic tree based on partial SSU rRNA gene sequences of *Cryptosporidium* spp.

### • Conclusions

The present study confirms the presence of *Cryptosporidium felis* infection in a group of cohabiting cats from a shelter environment. The combined use of rapid antigen testing and molecular analysis proved essential for accurate diagnosis. The findings highlight the importance of including cryptosporidiosis in the differential diagnosis of persistent diarrhea in cats. The genetic similarity with human-derived isolates underlines the potential public health relevance of *C. felis*. The study emphasizes the importance of proper hygiene, routine screening, and preventive veterinary care to reduce infection and transmission risk.