Molecular prevalence and subtyping of Cryptosporidium spp. in fecal samples collected from stray cats in İzmir, Turkey

Ahmet Efe Köseoğlu1, Hüseyin Can1,2*, Muhammet Karakavuk2,3, Mervenur Güvendi1, Aysu Değirmenci Döşkaya2,4, Pumla Bhekiwe Manyatsi1, Mert Döşkaya2,4, Adnan Yusıkel Gürüz2,4 and Cemal Ün1,2

Abstract

Background: Cryptosporidium spp. are obligate intracellular apicomplexan parasites transmitted to humans and other animals by contaminated water, food, or direct contact. They mainly cause gastrointestinal symptoms, although subclinical infections are also common. Cats are primarily infected by host-adapted Cryptosporidium felis while C. parvum and C. muris have also been detected in some cases. In this study, the molecular prevalence of Cryptosporidium spp. was investigated by screening 399 fecal samples collected from stray cats using nested PCR targeting the 18S rRNA gene for the first time in Turkey. Additionally, Cryptosporidium PCR-positive samples were genotyped by nested PCR-restriction fragment length polymorphism (RFLP), and subsequently, amplicons of 18S SSU rRNA were sequenced. They were further subtyped by amplification and sequencing of the gp60 gene.

Results: Among fecal samples screened, 12 of them (3%) were found to be Cryptosporium-positive, and according to RFLP and sequencing of 18S rRNA gene, all positive samples were identified as C. felis. Subtyping analyses at the gp60 gene showed that C. felis isolates belonged to the XIXa subtype family, which are closely related to human subtypes of the parasite.

Conclusions: The results of this study are important in terms of indicating the potential role of stray cats for transmission of Cryptosporidium spp. to humans or other animals. Also, the presence of XIXa, which is the dominant subtype family of C. felis in cats and humans was shown for the first time in stray cats of İzmir, Turkey.

Keywords: Cryptosporidium felis, 18S rRNA, gp60, XIXa, Cats, Turkey

Background

Cryptosporidium spp. are obligate intracellular apicomplexan parasites distributed worldwide and infecting all vertebrates including humans and domestic animals [1–3]. The main transmission routes of Cryptosporidium are consumption of contaminated water and food or direct contact with infected animals and humans [3]. In humans, Cryptosporidium causes gastrointestinal disturbance presented with diarrhea, although subclinical cases are also common. It may cause serious disseminated infections in immunocompromised individuals [3]. Cryptosporidium infection is generally asymptomatic in cats but symptoms like diarrhea may occur especially in young and newborn kittens. Additionally, the degeneration of host epithelial cells, loss of microvilli, and atrophy of the villi often occur in severe infections [4].

Among the 19 Cryptosporidium species (C. hominis, C. parvum, C. meleagridis, C. canis, C. felis, C. ubiquitum, ...
C. cuniculus, C. viatorum, C. muris, C. andersoni, C. erinacei, C. tyszleri, C. bovis, C. suis, C. scrofarum, C. occultus, C. xiaoai, C. fayeri and C. ditrichi) and 4 genotypes (Cryptosporidium chipmunk genotype I, mink genotype, skunk genotype and horse genotype), C. hominis and C. parvum are the most prevalently found in humans [3]. C. parvum is a zoonotic species whereas mainly anthropogenic C. hominis has been detected in different non-human animal species [5, 6]. Cryptosporidium canis, C. felis, C. meleagris, and C. ubiquitum are other significant zoonotic pathogens causing diseases in humans [3]. In cats, host-adapted C. felis is the main Cryptosporidium species, although C. parvum and C. muris have also been detected [7–12].

During the diagnosis of Cryptosporidium, microscopic staining methods such as Ziehl–Neelsen, Kinyoun, and Giemsa are used to identify parasite oocysts in the feces [7]. Antigen detection methods targeting the surface proteins of the parasite in stool samples are also used [13]. Microscopic and antigen detection methods are not useful for species and genotype identification due to morphologically identical oocysts and shared antigens [8].

Molecular methods including polymerase chain reaction (PCR), DNA sequencing, and restriction fragment length polymorphism (RFLP) based on the analysis of the small subunit 18S rRNA gene are used to detect the parasite DNA, and identify species and genotypes in animal, human, and environmental samples [7]. During the species identification and genotyping of Cryptosporidium spp. by an RFLP method, an 830-bp fragment of the 18S rRNA gene is digested using SspI and VspI restriction enzymes [14–16]. Additionally, a gene encoding 60-kDa glycoprotein (gp60) in the subtyping of C. parvum, C. hominis, C. meleagris, C. ubiquitum, C. viatorum, C. fayeri, C. ryanae, C. canis and C. felis has been used as a marker [3, 5, 17–20].

The study region İzmir harbors several stray cats that have close contact with humans and do not have sufficient veterinary care to prevent Cryptosporidium spp. transmission. Many important zoonotic parasites such as Toxoplasma gondii, Leishmania spp., and Blastocystis spp. have been detected in stray cats of İzmir in our previous studies [21–25]. This study initially investigated Cryptosporidium spp. in stray cats by a comprehensive PCR screening performed in 399 fecal samples. Secondly, species identification and subtyping analyses have been performed in Cryptosporidium-positive samples by RFLP and sequencing of the 18S rRNA and gp60 genes.

Results

**Molecular prevalence of Cryptosporidium spp.**

Cryptosporidium spp. DNA was detected in 12 of 399 fecal samples collected from stray cats by a nested PCR targeting the 18S rRNA gene. Accordingly, the molecular prevalence was 3%. Among the prevalence values of the regions, the highest prevalence value was observed in Karabağlar district with 4.5% which was followed by Narlıdere district with 3.4% (Fig. 1).

**Species identification**

Species identification of Cryptosporidium PCR-positive samples was performed by two different approaches including RFLP and Sanger sequencing analyses. According to the results, all Cryptosporidium-positive samples were identified as C. felis. After digestion with SspI, two fragments 426 bp and 390 bp were detected for all C. felis positive samples. Three fragments 444 bp, 247 bp, and 106 bp were detected for C. parvum which was used as the positive control. Sequencing of 18S rRNA gene also confirmed that all Cryptosporidium-positive samples were C. felis (Table 1). Similarity rates of C. felis samples identified in this study varied between 91 and 98% compared to reference C. felis isolates deposited in GenBank (Table 1).

**Subtyping of C. felis**

Subtypes of C. felis isolates were identified by Sanger sequencing of the gp60 gene. The scheme defined by Jiang et al., (2020) [26] was used for subtyping. The relation of subtypes was shown by phylogenetic analysis performed with reference C. felis isolates subtyped as XIXa, XIXb, XIXc, XIXd, or XIXe. According to this scheme, a repeat sequence with one copy called R1 (CCACCTAGTGCCGGTACGTGCTCCCCTGCT) and a 9 bp deletion were detected in all C. felis isolates. Also, two indels, which are located between positions 706-711 bp, and 1015–1068 bp, were not available. Another repeat sequence called R2 (AGACCAACTACGCTACGCG AGCAGCTAGGTGCTGACAGAGA) was detected as one or two copies whereas the GGT sequence among positions 1156 and 1167 was detected as two, three, or four copies. These findings were compatible with the subtype family named as XIXa (Table 2). Also, all C. felis samples were clustered within the subtype family XIXa in the phylogenetic tree (Fig. 2).

**Discussion**

Cats are reservoirs of different infectious diseases caused by parasites or bacteria with zoonotic potential [27–29]. To date, the presence of important zoonotic parasites such as T. gondii, Blastocystis spp., and Leishmania spp. in stray cats living in İzmir (our study region) have been reported using molecular or serological methods with a prevalence of 14.4%, 3.7%, and 11.1%, respectively [22, 23, 25]. Although different Cryptosporidium species have been detected in cats, which play a role as reservoir host,
the prevalence of Cryptosporidium spp., as well as species and subtypes in stray cats of İzmir are not known in detail. However, the prevalence of Cryptosporidium in humans has been frequently investigated by microscopy or immunological methods rather than molecular methods that enable species identification or subtyping in İzmir. According to obtained results, although the prevalence of Cryptosporidium spp. changed based on the sample number analyzed or method used, high prevalence rates varying from 33.5% to 37.4% were detected in İzmir [30, 31].

In this comprehensive study, three significant findings have been reported for the first time in Turkey. Firstly, the molecular prevalence of Cryptosporidium spp. was found to be 3% (12/399) in stray cats. Although this prevalence is low, it is rather important in terms of indicating that stray cats can act as a potential source of human and other animal infections in İzmir, Turkey.

Fig. 1 The map shows the prevalence values among different regions of İzmir, Turkey. Datawrapper (https://www.datawrapper.de/) was used to create the map
Secondly, all Cryptosporidium-positive samples were identified as *C. felis* by nested PCR–RFLP and Sanger sequencing. *Cryptosporidium felis* causes infections in cats and in some cases, in cows and humans as well [1, 3, 32]. Human cryptosporidiosis cases caused by *C. felis* have been detected frequently in developing countries [2, 17, 33–36]. However, there are uncertainties about the zoonotic transmission from cats into humans because of the limited number of cases and lack of sufficient information about *C. felis* isolated from cats at the genetic level [17]. In line with this, in a study investigating household transmission of zoonotic *Cryptosporidium* spp. from pet dogs and cats to their owners, it was emphasized that domestic cats do not play an important role as the source of human cryptosporidiosis [37].

The molecular prevalence of *C. felis* in cats varied from 0.3% to 11% in studies conducted in different regions (Table 3). Similarly, *C. felis* was detected in humans using molecular methods with different prevalence rates ranging from 0.4% to 4.5% (Table 3). Although any prevalence study associated with cryptosporidiosis in cats except our study showing a 3% *C. felis* prevalence has not been conducted in Turkey, a recent study identified for the first time a *C. felis* isolate (ANK_1) (NCBI accession number: MN394123) by molecular methods in a house cat [38]. Additionally, *C. felis* has been identified in surface water samples in the province of Samsun, Turkey [39]. This finding is relevant because it highlights the capability of infected cats of contaminating the environment (soil, waters) with their feces.

In subtyping studies based on gp60 gene variations of *C. felis*, five subtype families (XIXa, XIXb, XIXc, XIXd,

| Table 1 | BLAST results of *C. felis* isolates based on 18S rRNA gene
| Sample | Accession number | Percentage of nucleotide identity |
|--------|-----------------|----------------------------------|
| *C. felis* cat isolate 1 | KJ194110.1 | 725/780 (93%) |
| *C. felis* cat isolate 2 | AB694730.1 | 468/491 (95%) |
| *C. felis* cat isolate 3 | AB694730.1 | 658/722 (91%) |
| *C. felis* cat isolate 4 | KJ194110.1 | 780/795 (98%) |
| *C. felis* cat isolate 5 | KJ194110.1 | 774/794 (97%) |
| *C. felis* cat isolate 6 | KJ194110.1 | 771/797 (97%) |
| *C. felis* cat isolate 7 | KJ194110.1 | 768/806 (95%) |
| *C. felis* cat isolate 8 | KJ194110.1 | 797/813 (98%) |
| *C. felis* cat isolate 9 | MH115431.1 | 743/780 (95%) |
| *C. felis* cat isolate 10 | MK982512.1 | 734/784 (94%) |
| *C. felis* cat isolate 11 | AF159113.1 | 766/818 (94%) |
| *C. felis* cat isolate 12 | AF159113.1 | 716/769 (93%) |

| Table 2 | Subtyping of *C. felis* isolates using reference positions based on gp60 gene
| Sample | R1 (463–556) | Deletion (667–679) | Indel (706–711) | R2 (770–910) | Indel (1015–1068) | Indel (1156–1167) | Subtype family |
|--------|--------------|-----------------|-----------------|--------------|-----------------|-----------------|----------------|
| *C. felis* cat isolate 1 | 1 copy | 9 bp | - | 2 copy | - | 4 GGT | XIXa |
| *C. felis* cat isolate 2 | 1 copy | 9 bp | - | 1 copy | - | a | XIXa |
| *C. felis* cat isolate 3 | 1 copy | 9 bp | - | 2 copy | - | a | XIXa |
| *C. felis* cat isolate 4 | 1 copy | 9 bp | - | 2 copy | - | a | XIXa |
| *C. felis* cat isolate 5 | 1 copy | 9 bp | - | 2 copy | - | 3 GGT | XIXa |
| *C. felis* cat isolate 6 | 1 copy | 9 bp | - | 1 copy | - | 4 GGT | XIXa |
| *C. felis* cat isolate 7 | 1 copy | 9 bp | - | 1 copy | - | 4 GGT | XIXa |
| *C. felis* cat isolate 8 | 1 copy | 9 bp | - | 1 copy | - | 2 GGT | XIXa |
| *C. felis* cat isolate 9 | 1 copy | 9 bp | - | 2 copy | - | 3 GGT | XIXa |
| *C. felis* cat isolate 10 | 1 copy | 9 bp | - | 2 copy | - | 2 GGT | XIXa |
| *C. felis* cat isolate 11 | 1 copy | 9 bp | - | 2 copy | - | 4 GGT | XIXa |
| *C. felis* cat isolate 12 | 1 copy | 9 bp | - | 1 copy | - | 4 GGT | XIXa |
| Reference isolate 1 | 1–3 copies | 3 or 9 bp | - | 1–4 copies | - | 2–5 GGT | XIXa |
| Reference isolate 2 | 1 or 2 copies | 6 or 15 bp | - | 2–4 copies | 18 bp | 4 GGT | XIXb |
| Reference isolate 3 | 2 copies | 15 bp | - | 3 copies | - | 3 GGT | XIXc |
| Reference isolate 4 | 1 copy | - | - | 1 copy | - | 4 GGT | XIXd |
| Reference isolate 5 | 1 copy | - | - | 1 copy | - | 4 GGT | XIXe |

Reference positions are from Jiang et al. 2020 [26]
* shows the unevaluated sequences because of sequencing errors
R1: 33-bp tandem repeat (CCACCTAGTGCCGGTATGGCTATCCCCCTGCT)
R2: 39-bp tandem repeat (AGCAACAATACGGCTACAGCGAGCAGTGGAGTCGACA)
and XIXe) have been identified using 200 *C. felis* isolates from all around the world [17]. Among the five subtype families, the XIXa subtype family has been reported as the dominant subtype family in cats and humans in previous studies [17, 26, 52]. The third important finding in our study is the detection of subtype family XIXa in stray cats, which is consistent with previous results (Table 2; Fig. 2). This result also indicates that the XIXa subtype family is the dominant subtype in circulation causing the transmission between stray cats and humans in İzmir.
Conclusion

The presence of *C. felis* in stray cats was demonstrated for the first time by analyzing a huge number of fecal samples in Turkey. Detection of the dominant XIXa subtype family in cats and humans indicated the possible transmission risk between stray cats and humans in İzmir. Accordingly, it was thought that the stool of stray cats should be analyzed for the presence of *C. felis* and positive stray cats should be treated to reduce the zoonotic transmission from cats to humans by the municipality veterinarians during their routine testing before sterilization operation.

Materials and methods

Fecal samples

Fecal samples (*n* = 399) collected from stray cats which were brought for routine surgical sterilization process performed by the Veterinary clinics of the Municipality of İzmir were included in this study. Regions that fecal samples were collected were Konak (*n* = 159), Narlıdere (*n* = 148), Çiğli (*n* = 70) and Karabağlar (*n* = 22) districts. Before surgical sterilization and receiving any treatment, the fecal samples were collected from stray cats which were kept in separate cages.

Nested PCR–RFLP

DNA was extracted by a commercial stool DNA isolation kit (RTA Labs, Turkey) according to the manufacturer's instructions. During DNA isolation, 100 mg of fecal sample was used, and they were eluted with 100 μl elution buffer [23]. A nested PCR targeting small subunit 18S rRNA gene was applied as described before [14]. In the initial reaction, 5'-TTC TAG AGC TAA TAC ATG CG-3' and 5'-CCC TAA TCC TTC GAA ACA GGA-3' primers were used to amplify a 1325 bp gene fragment. In the second reaction, 5'-GGA AGG GTT GTA TTT ATT AGA TAA AG-3' and 5'-AAG GAG TAA GGA ACA ACC TCCA-3' primers were used to amplify gene fragments with a molecular size approximately 826–864 bp from the initial reaction product. In the initial reaction of nested PCR, 100 μl amplification reaction included 2 μl template DNA, 2 μl primers, 16 μl MgCl₂ (25 nM), 2 μl dNTP, 10 μl Taq buffer, and 0,5 μl Taq DNA Polymerase (ThermoFisher). In the second step of nested PCR, 4 μl PCR product as template DNA and 4 μl MgCl₂ (25 nM) was used as different from the first step. The nested PCR was performed using the following protocol for both steps: 3 min initial denaturation step at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 55 °C, and 1 min at 72 °C, and a final extension of 7 min at 72 °C. After amplification, PCR products

| Molecular prevalence | Target gene | Subtyping gene | Subtype family | Host | Country | Reference |
|----------------------|-------------|----------------|---------------|------|---------|-----------|
| 2.0% (7/357)         | 18S rRNA    | -              | -             | household cats | Japan    | [40]      |
| 0.3% (1/329)         | 18S rRNA    | -              | -             | pet shop kittens | Japan    | [40]      |
| 2.5% (2/80)          | 18S rRNA    | -              | -             | cats | Thailand | [41]      |
| 4.5% (7/155)         | 18S rRNA    | -              | -             | HIV-infected patients | Thailand | [42]      |
| 1.7% (18/1063)       | actin       | -              | -             | cats | Australia | [43]      |
| 11% (5/46)           | 18S rRNA    | -              | -             | cats | Colombia | [44]      |
| 5.4% (3/55)          | 18S rRNA    | -              | -             | cats | Brazil   | [45]      |
| 4.8% (12/250)        | 18S rRNA    | -              | -             | domestic cats | Mississippi and Alabama | [46] |
| 1.92% (1/52)         | 18S rRNA    | -              | -             | cats | China    | [10]      |
| 5% (21/418)          | 18S rRNA    | -              | -             | cats | China    | [47]      |
| 0.49% (3/609)        | 18S rRNA    | -              | -             | humans | China    | [48]      |
| 0.35% (1/283)        | 18S rRNA    | -              | -             | immunodeficient patients | Poland | [49]      |
| 3.3% (1/30)          | 18S rRNA    | -              | -             | humans | Sweden   | [50]      |
| 0.9% (1/108)         | 18S rRNA    | gp60           | XIXa          | patients | Mozambique | [51] |
| 1% (4/398)           | 18S rRNA    | gp60           | XIXa          | pet shelter/shop cats, stray cats | China | [17] |
| -                    | 18S rRNA    | gp60           | XIXa          | cats | Peru, USA, Slovakia, Australia | [26] |
| -                    | 18S rRNA    | gp60           | XIXa, XIXc, XIXd, XIXe | humans | Peru, USA, Jamaica, Portugal, Nigeria, China, Kenya | [26] |

Table 3 Molecular prevalence and subtyping data of *C. felis* isolates detected in cats and humans from different countries

DNA was extracted by a commercial stool DNA isolation kit (RTA Labs, Turkey) according to the manufacturer’s instructions. During DNA isolation, 100 mg of fecal sample was used, and they were eluted with 100 μl elution buffer [23]. A nested PCR targeting small subunit 18S rRNA gene was applied as described before [14]. In the initial reaction, 5’-TTCTAGAGCTAAATCGTCCG-3’ and 5’-CCTAATCTTTCGAAACACGA-3’ primers were used to amplify a 1325 bp gene fragment. In the second reaction, 5’-GGAAGGTGTGTATTTATTAGATAAAG-3’ and 5’-AAGGATACGAAACACCTCCA-3’ primers were used to amplify gene fragments with a molecular size approximately 826–864 bp from the initial reaction product. In the initial reaction of nested PCR, 100 μl amplification reaction included 2 μl template DNA, 2 μl primers, 16 μl MgCl₂ (25 nM), 2 μl dNTP, 10 μl Taq buffer, and 0,5 μl Taq DNA Polymerase (ThermoFisher). In the second step of nested PCR, 4 μl PCR product as template DNA and 4 μl MgCl₂ (25 nM) was used as different from the first step. The nested PCR was performed using the following protocol for both steps: 3 min initial denaturation step at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at 55 °C, and 1 min at 72 °C, and a final extension of 7 min at 72 °C. After amplification, PCR products
were visualized using 1% agarose gel. Then, PCR products were purified by Qiaquick PCR Purification Kit (Qiagen, USA), sequenced, and compared with the NCBI database (https://www.ncbi.nlm.nih.gov) by BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For sequencing of the 18S rRNA gene, forward primer (5'-GGAAGGGTTGATTTTATTAGATAAAG-3') was used.

For species identification of Cryptosporidium-positive samples, PCR products with a size of 826–864 bp were digested by SspI restriction enzyme (ThermoFisher) for 4 h [14–16]. During digestion, 20 μl total reaction volume included 10 μl amplified PCR product, 1.5 μl restriction enzyme, 2 μl restriction buffer (10x), and 6.5 μl distilled water. After digestion, PCR products were visualized using 2.5% agarose gel electrophoresis. After digesting by SspI enzyme, the expected product sizes for C. felis are 426 bp and 390 bp whereas [2, 16] the expected products for C. parvum are 444 bp, 247 bp, and 106 bp [16].

Subtyping
Cryptosporidium felis isolates were subtyped by sequencing of the gp60 gene as described previously [5]. In the initial reaction, GP60CF_F1 (5'-TTTCCGTTATGGTTGCAGTTGCA-3') and GP60CF_R1 (5'-ATCGGATCCCCACCATCGAAC-3') primers were used to amplify a 1200 bp gene fragment. In the second reaction, GP60CF_F2 (5'-GGCCCCTCTGAAGGATGTA-3') and GP60CF_R2 (5'-CGGTGTTCTCCTGATGCTC-3') primers were used to amplify a 900 bp gene fragment from the initial reaction product. In the initial reaction of nested PCR, 20 μl amplification reaction included 3 μl template DNA, 0.5 μl primers, and 12.5 μl Dye Master Mix II (GeneMark). The second step of nested PCR was the same as the first step except the 1 μl PCR product that was used as template DNA. The nested PCR was performed using the following protocol for both steps: 4 min initial denaturation step at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 1.5 min at 72 °C, and a final extension of 7 min at 72 °C. Obtained PCR products were visualized using 1% agarose gel. Then, PCR products purified by Qiaquick PCR Purification Kit (Qiagen, USA) were sequenced, and generated sequences belonging to the gp60 gene were aligned with reference sequences subtyped as XIXa, XIXb, XIXc, XIXd, or XIXe by Jiang et al., 2020. For sequencing the gp60 gene, forward primer (5'-GGGGCTCTGAAGGATGTA-3') was used. Subtype determination was performed by a scheme defined by Jiang et al. [26]. Next, a phylogenetic tree was constructed by the Maximum Likelihood method using the Kimura-3 parameter with 1000 bootstraps in order to confirm subtype results.

Abbreviations
C. felis: Cryptosporidium felis; PCR: Polymerase chain reaction; gp60: 60-KDa glycoprotein gene; RFLP: Restriction fragment length polymorphism; 18S rRNA: Small subunit rRNA gene.

Acknowledgements
Not Applicable.

Authors’ contributions
Conceptualization: HC, and CÜ; Methodology: HC, and CÜ. Formal analysis and investigation: AEK, MG, PBM, HC, MD, AED and MK; Writing-original draft preparation: HC. Writing-review and editing: HC, CÜ, MD, ADD and AVG. Funding acquisition: HC. Supervision: HC, and CÜ. All authors read and approved the final manuscript.

Funding
This study was supported by a project given by the Ege University Scientific Research Projects Coordination Unit (Project number: TGA-2021–22513) to H.C.

Availability of data and materials
All sequences obtained from 18S rRNA and gp60 genes of Cryptosporidium isolates were deposited into GenBank (National Center for Biotechnology Information Search database) under accession numbers OL615014–OL615025 (for the 18S rRNA gene) and OL631626–OL631617 (for gp60).

Declarations
Ethics approval and consent to participate
The protocol for collecting fecal samples from stray cats was declared as exempt according to the instructions and approval of the Institutional Animal Care and Use Committee (IACUC) of Ege University. All methods were performed in accordance with the relevant guidelines and regulations. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication
Not applicable.

Competing interest
The authors declare that they have no competing interests.

Author details
1 Faculty of Science Department of Biology Molecular Biology Section, Ege University, İzmir, Turkey. 2 Faculty of Medicine Department of Parasitology, Ege University, İzmir, Turkey. 3 Ege University Ödemiş Technical Training College, İzmir, Turkey. 4 Faculty of Medicine Department of Parasitology, Ege University, İzmir, Turkey.

Received: 27 September 2021   Accepted: 28 February 2022
Published online: 07 March 2022

References
1. Huber F, da Silva S, Bomfim TC, Teixeira KR, Bello AR. Genotypic characteriza-
2. Ciełoszyk J, Goni P, García A, Remacha MA, Sánchez E, Clavel A. Two cases of zoonotic cryptosporidiosis in Spain by the unusual species Crypto-
3. Ryan U, Zahedi A, Feng Y, Xiao L. An Update on Zoonotic Cryptosporid-
4. Scorza V, Tangtrongsup S. Update on the diagnosis and management of Crypto-
5. Rojas-Lopez L, Elwin K, Chalmers RM, Enemark HL, Besser J, Trelle K. Devel-

Not Applicable.

Acknowledgements

Not Applicable.

Author details
1 Faculty of Science Department of Biology Molecular Biology Section, Ege University, İzmir, Turkey. 2 Faculty of Medicine Department of Parasitology, Ege University, İzmir, Turkey. 3 Ege University Ödemiş Technical Training College, İzmir, Turkey. 4 Faculty of Medicine Department of Parasitology, Ege University, İzmir, Turkey.

Received: 27 September 2021   Accepted: 28 February 2022
Published online: 07 March 2022

References
1. Huber F, da Silva S, Bomfim TC, Teixeira KR, Bello AR. Genotypic characteri-
2. Ciełoszyk J, Goni P, García A, Remacha MA, Sánchez E, Clavel A. Two cases of zoonotic cryptosporidiosis in Spain by the unusual species Crypto-
3. Ryan U, Zahedi A, Feng Y, Xiao L. An Update on Zoonotic Cryptosporid-
4. Scorza V, Tangtrongsup S. Update on the diagnosis and management of Crypto-
5. Rojas-Lopez L, Elwin K, Chalmers RM, Enemark HL, Besser J, Trelle K. Devel-

Not Applicable.

Acknowledgements

Not Applicable.
6. Widmer G, Koster PC, Carmina D. Cryptosporidium hominis infections in non-human animal species: revisiting the concept of host specificity. Int J Parasitol. 2020;50(4):253–62.

7. Alves MEM, Martins FDC, Braunig P, Pivotta FL, Sangioni LA, Vogel FSF. Molecular detection of Cryptosporidium spp. and the occurrence of intestinal parasites in fecal samples of naturally infected dogs and cats. Parasitol Res. 2018;117:3033–8.

8. Lucio-Foster A, Griffiths JK, Cama VA, Xiao L, Bowman DD. Minimal zoozotic risk of cryptosporidiosis from pet dogs and cats. Trends Parasitol. 2010;26(4):174–9.

9. Yang R, Ying JL, Monis P, Ryan U. Molecular characterisation of Cryptosporidium and Giardia in cats (Felis catus) in Western Australia. Exp Parasitol. 2015;155:13–8.

10. Li W, Li Y, Song M, Lu Y, Yang J, Tao W, Jiang Y, Wan Q, Zhang S, Xiao L. Molecular prevalence and subtype characterization of Cryptosporidium and Giardia duodeni in cats and dogs in Heilongjiang province, China. Vet Parasitol. 2015;208(1–3):125–34.

11. Gil H, Cano L, de Lucia A, Balbo B, de Mingó MH, Cardona GA, Fernández-Basteria JA, Asambrun-Garín J, López-Molina N, Carmina D. Detection and molecular diversity of Giardia duodeni and Cryptosporidium spp. in sheltered dogs and cats in Northern Spain. Infect Genet Evol. 2017;50:62–9.

12. Li W, Liu X, Gu Y, Jiang W, Zhao Q, Zhang S, Xiao L. Prevalence and genetic characteristics of Cryptosporidium, Enterocytotoxin-bieneusi and Giardia duodenalis in cats and dogs in Heilongjiang province, China. Vet Parasitol. 2015;208(1–3):125–34.

13. Gil H, Cano L, de Lucia A, Balbo B, de Mingó MH, Cardona GA, Fernández-Basteria JA, Asambrun-Garín J, López-Molina N, Carmina D. Detection and molecular diversity of Giardia duodeni and Cryptosporidium spp. in sheltered dogs and cats in Northern Spain. Infect Genet Evol. 2017;50:62–9.

14. Xiao L, Escalante L, Yang C, Sulaiman I, Escalante AA, Montali RJ, Murti YP, Voltz C. Subtyping Cryptosporidium ryanae: A common pathogen in bovine hosts. Exp Parasitol. 2009;121(3):219–23.

15. Xiao L, Bern C, Limor I, Sulaiman I, Roberts J, Checkley W, Cabrera L, Gil-Moreno S. Molecular genotyping and sub-genotyping of Cryptosporidium felis isolates from different marsupial hosts. Exp Parasitol. 2009;121(3):219–23.

16. Das P, Roy SS, MitraDhar K, Dutta P, Bhattacharya MK, Sen A, Ganguly S, Bhattacharya SK, Lal AA. Phylogenetic analysis of Cryptosporidium parasites based on the small-subunit rRNA gene locus. Appl Environ Microbiol. 1999;65(4):1578–83.

17. Xiao L, Bern C, Limor I, Sulaiman I, Roberts J, Checkley W, Cabrera L, Gil-Moreno S. Molecular genotyping and sub-genotyping of Cryptosporidium felis isolates from different marsupial hosts. Exp Parasitol. 2009;121(3):219–23.

18. Power ML, Cheung-Kwok-Sang C, Slade M, Williamson S. Cryptosporidium and Giardia duodenalis assemblage F and Cryptosporidium felis in asymptomatic adult cattle in Northern Spain. Vet Parasitol. 2015;209(3–4):258–63.

19. de Lucio A, Merino FJ, Martinez-Ruiz R, Balbo B, Aguilera M, Fuentes I, Carmina D. Molecular genotyping and sub-genotyping of Cryptosporidium spp. isolates from symptomatc individuals attending two major public hospitals in Madrid. Spain Infect Genet Evol. 2016;37:49–56.

20. de Lucio A, Balbo B, Aguilera M, Cardona GA, Fernández-Crespo JC. Genotyping of Cryptosporidium felis feline isolates. Pathogens. 2020;9(1):2446–54.

21. Ito Y, Itoh N, Iijima Y, Kimura Y. Subtype distribution of zoonotic pathogen Cryptosporidium felis in cats of İzmir, Turkey: First report of “ST4 allele 42” in cats. Pol J Vet Sci. 2021;24:217–23.

22. Jiang W, Roellig DM, Lebbad M, Beser J, Troell K, Guo Y, Li N, Xiao L, Feng Y. Subtype distribution of zoonotic pathogen Cryptosporidium felis in humans and animals in several countries. Emerg Microbes Infect. 2020;9(1):2446–54.

23. Gerhold RW, Jessup DA. Zoonotic diseases associated with free-roaming cats. Zoonoses Public Health. 2013;60(3):189–95.

24. Diakou A, Di Cesare A, Accettura PM, Barros L, Iorio R, Pascoli B, Di Francote Regalbono A, Halos L, Beugnet F, Traversa D. Intestinal parasites and vector-borne pathogens in stray and free-roaming cats living in continental and insular Greece. PLoS Negl Trop Dis. 2017;11(11):e005335.

25. Kwak D, Seo MG. Genetic Analysis of Zoonotic Gastrointestinal Protozoa and Microsporidia in Shelter Cats in South Korea. Pathogens. 2020;9(11):894.

26. Turgay N, Unver-Yolasizmaz A, Cuyr T, Ozem SB, Tos Z. Monthly distribution of intestinal parasites detected in a part of western Turkey between May 2009-April 2010 results of acid fast and modified trichrome staining methods. Turkiye Parazitol Derg. 2012;36:741–7.

27. Ulusan O, Zorbozan O, Yetismis K, Tos Z, Unver N. Distribution of the intestinal parasites detected in Ege University Medical Faculty Parasitology Department for 2-year evaluation, Turkey. Mikrobiyoloji Cem Derg. 2019;49:86–91.

28. de Lucio A, Merino FJ, Martinez-Ruiz R, Balbo B, Aguilera M, Fuentes I, Carmina D. Molecular genotyping and sub-genotyping of Cryptosporidium spp. isolates from symptomatc individuals attending two major public hospitals in Madrid. Spain Infect Genet Evol. 2016;37:49–56.

29. de Lucio A, Balbo B, Aguilera M, Cardona GA, Fernández-Crespo JC. Molecular genotyping and sub-genotyping of Cryptosporidium felis isolates from pet dogs and cats in the province of Álava, Northern Spain Acta Trop. 2017;170:448–56.

30. Sursal N, Simsek E, Yildiz K. Occurrence and First Molecular Characterization of Cryptosporidium felis in a Cat in Turkey. Kafkas Univ Vet Fak Derg. 2020;26:833–7.

31. Koloren Z, Ayaz E. Genotyping of Cryptosporidium spp. in environmental water in Turkey. Acta Parasitol. 2016;61(6):671–9.

32. Ito Y, Itoh N, Iijima Y, Kimura Y. Molecular prevalence of Cryptosporidium species among household cats and pet kittens in Japan. JFMS Open Rep. 2017;3(2):205511697730719.

33. Koopmann K, Mori H, Thammasonthijareen P, Prasertbun R, Pintong AP, Popuk S, Rojekittikhun W, Chaisiri K, Sulthanka Y, Mahittikorn A. Molecular identification of Cryptosporidium spp. in seagulls, pigeons, dogs, and cats in Thailand. Parasite. 2014;21:152.

34. Sannella AR, Suputtamongkol Y, Wongsawat E, Cacciò SM. A retrospective molecular study of Cryptosporidium species and genotypes in HIV-infected patients from Thailand. Parasit Vectors. 2019;12:1–6.

35. Palmer CS, Traub RJ, Robertson JD, Devlin G, Rees R, Thompson RC. Determining the zoonotic significance of Giardia and Cryptosporidium in Australian dogs and cats. Vet Parasitol. 2008;154(1–2):142–7.

36. Santin M, Trout JM, Vecino JA, Dubey JP, Rayer F. Cryptosporidium, Giardia and Enterocytotoxin-bieneusi in cats from Bogota (Colombia) and genotyping of isolates. Vet Parasitol. 2006;141(3–4):334–9.

37. de Oliveira AGL, Sudré AP, do Bergamo Bomfim TC, Santos HLC. Molecular characterization of Cryptosporidium spp. in dogs and cats in the city of Rio de Janeiro, Brazil, reveals potentially zoonotic species and genotype. PLoS One. 2021;16:e0255087.
46. Ballweber LR, Panuska C, Huston CL, Vasilopulos R, Pharr GT, Mackin A. Prevalence of and risk factors associated with shedding of Cryptosporidium felis in domestic cats of Mississippi and Alabama. Vet Parasitol. 2009;160:306–10.

47. Li J, Dan X, Zhu K, Li N, Guo Y, Zheng Z, Feng Y, Xiao L. Genetic characterization of Cryptosporidium spp. and Giardia duodenalis in dogs and cats in Guangdong, China. Parasit Vectors. 2019;12(1):571.

48. Wang T, Wei Z, Zhang Y, Zhang Q, Zhang L, Yu F, Qi M, Zhao W. Cryptosporidium in asymptomatic children in Southern Xinjiang, China and the potential of zoonotic transmission. 2021. (Res. Sq).

49. Bednarska M, Jankowska I, Pawelas A, Piwczyńska K, Bajer A, Wolska-Kuśnierz B, Wielopolska M, Wielopolski W, Fiałcik R. Prevalence of Cryptosporidium, Blastocystis, and other opportunistic infections in patients with primary and acquired immunodeficiency. Parasitol Res. 2018;117:2869–79.

50. Gherasim A, Lebbad M, Insulander M, Decraene V, Kling A, Hjertqvist M, Wallensten A. Two geographically separated food-borne outbreaks in Sweden linked by an unusual Cryptosporidium parvum subtype, October 2010. Euro Surveill. 2012;17(40):20318.

51. Casmo V, Lebbad M, Maungate S, Lindh J. Occurrence of Cryptosporidium spp. and Cystoisospora belli among adult patients with diarrhoea in Maputo, Mozambique. Helminthology. 2018;4:e00769.

52. Lebbad M, Windholtz M, Skovgaard-Crothers C, Stensvold CR, Beser J. High Diversity of Cryptosporidium Species and Subtypes Identified in Cryptosporidiosis Acquired in Sweden and Abroad. Pathogens. 2021;10:523.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.