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Case Report

Occurrence and First Molecular Characterization of *Cryptosporidium felis* in a Cat in Turkey

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Abstract

Cryptosporidium species are highly prevalent and significant zoonotic protozoan parasites that cause severe diarrhea in many hosts. To date, cryptosporidiosis has been molecularly reported from humans and different animal hosts in Turkey. The aim of this case report is to reveal the molecular characterization based on small subunit (SSU) rRNA gene sequence of *Cryptosporidium* oocysts that were microscopically found in a three-month age female cat with severe diarrhea. SSU rRNA gene of *Cryptosporidium* was amplified for sequence and phylogenetic analyses. According to sequence analysis, *Cryptosporidium* spp. oocysts were characterized as *C. felis* (isolate name: ANK_1; accession number: MN394123). ANK_1 isolate showed a range from 99.1% to 100% identity with the most of *C. felis* sequences available in GenBank. However, it also exhibited high genetic distance (1.5-4.3%) with some of *C. felis* sequences. The intraspecific genetic differences among all SSU rRNA sequences of *C. felis* isolates included in the data set were determined ranged from 0.0% to 6.9%. As a conclusion, we provided the first microscopic and molecular evidence of the occurrence of *C. felis* in a house cat in Turkey. Although *C. felis* has a relatively low zoonotic risk to public health when compared with *C. parvum,* infected cats should not be neglected for zoonotic transmission due to close relationships between cats and humans.

Keywords: *Cryptosporidium felis, Cat, Molecular characterization, SSU rRNA gene, Turkey*

Türkiye'de Bir Kedide *Cryptosporidium felis*'in Varlığı ve İlk Moleküler Karakterizasyonu

Öz

Cryptosporidium türleri, birçok konakta ciddi ishale neden olan oldukça yaygın ve önemli zoonotik protozoan parazitlerdir. Bugüne kadar cryptosporidiosis, Türkiye'de insanlarda ve farklı hayvan konaklarında moleküler olarak rapor edilmiştir. Bu olgu sunumunun amacı, ağır ishalli üç aylık dişi bir kedide mikroskobik olarak saptanan *Cryptosporidium* ookistlerinin small subunit (SSU) rRNA gen sekansına dayanan moleküler karakterizasyonunu ortaya koymaktır. *Cryptosporidium*'un SSU rRNA geni, sekans ve filogenetik analizler için amplifiye edilmiştir. Sekans analizi sonucu, *Cryptosporidium* spp. ookistleri *C. felis* (izolat adı: ANK_1; erişim numarası: MN394123) olarak karakterize edilmiştir. ANK_1 izolatı, GenBank'ta mevcut *C. felis* sekanslarının birçoğuyla %99,1 ile %100 arasında benzerlik göstermiştir. Ancak, bazı *C. felis* sekanslarıyla ise yüksek düzeyde genetik farklılık (%1.5-4.3) göstermiştir. Veri setine dahil edilen tüm *C. felis* izolatlarının SSU rRNA sekansları arasındaki tür içi genetik farklılık %0.0 ile %6.9 arasında belirlenmiştir. Sonuç olarak, bu olgu sunumu ile Türkiye'de bir ev kedisinde *C. felis*'in varlığı mikroskobik ve moleküler olarak ilk kez gösterilmiştir. *Cryptosporidium felis, C. parvum*'a kıyasla halk sağlığı açısından nispeten daha az zoonotik risk taşımasına rağmen, insanlar ve kediler arasındaki yakın ilişkiler dikkate alındığında enfekte kedilerin zoonotik bulaşmada önemli olabileceği göz ardı edilmemelidir.

Anahtar sözcükler: *Cryptosporidium felis, Kedi, Moleküler karakterizasyon, SSU rRNA geni, Türkiye*

INTRODUCTION

Cryptosporidium species are significant apicomplexan parasites that infect the gastrointestinal system of numerous hosts (mammals, birds, reptiles, and fishes), including humans [1,2]. To date, 38 *Cryptosporidium* species have been recognized [3]. Cats are commonly infected with *C. felis* [4-6]. In addition, *C. parvum* [6,7], *C. muris* [4], *C. ryanae* [4], and *Cryptosporidium* rat genotype III-IV [4,8] species have also been reported from cats.

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Most of the cats infected with *Cryptosporidium* spp. are usually asymptomatic. Diarrhea occurs more frequently in young and newborn kittens [9,10]. Feces are usually watery and do not contain mucus, blood, and melena. Other common clinical signs of cryptosporidiosis in cats are anorexia and weight loss [11].

Different diagnostic techniques including the microscopic examination (wet mount preparation, staining methods such as modified acid-fast stain or fluorescent stains), histological examination, immunological and various molecular methods are available for detection of cryptosporidiosis [12]. Molecular characterization of *Cryptosporidium* species is important and necessary for precise identification of organisms and to understand the zoonotic transmissions [13]. PCR-RFLP and DNA sequencing are the most frequently used assays for molecular identification and characterization of *Cryptosporidium* species. The small subunit (SSU) rRNA gene is commonly preferred for genotyping *Cryptosporidium* in many hosts and environmental samples [14].

Cryptosporidium infections have been reported from humans and different animal hosts including cats in Turkey [15-21]. It was aimed to reveal the first molecular characterization based on SSU rRNA gene sequence of *Cryptosporidium* isolate found in a cat in this case report.

CASE HISTORY

A fecal sample obtained from a mixed-breed, three-month old female cat with severe diarrhea was sent to Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology Laboratory for parasitological examination by a veterinary clinic in June 2019. According to anamnesis, despite the long-term antibiotic treatment, no reduction in the severity of diarrhea was observed. It was also informed that the cat mostly lived in a house and had also access to the garden of the house. Written informed consent of the cat's owner was obtained for using the data in scientific publications.

Firstly, fecal consistency scores were evaluated according to the modified fecal scoring system [22] and determined as 6 (watery and no texture, occurs as puddles). After routine parasitological examination, the fecal sample was stained with carbol-fuchsin dye (CF) to detect *Cryptosporidium* oocysts [23]. *Cryptosporidium* spp. oocysts (~5 µm diameter) in the fecal sample stained with CF dye were visualized as bright white color on the red background using the light microscope (Olympus BX43 Tokyo, Japan equipped with Olympus DP73 digital camera) *(Fig. 1)*. No other parasites were found in the fecal sample.

For advanced molecular diagnosis, the genomic DNA (gDNA) was extracted from the fecal sample using a QIAamp Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Nested-PCR was performed to

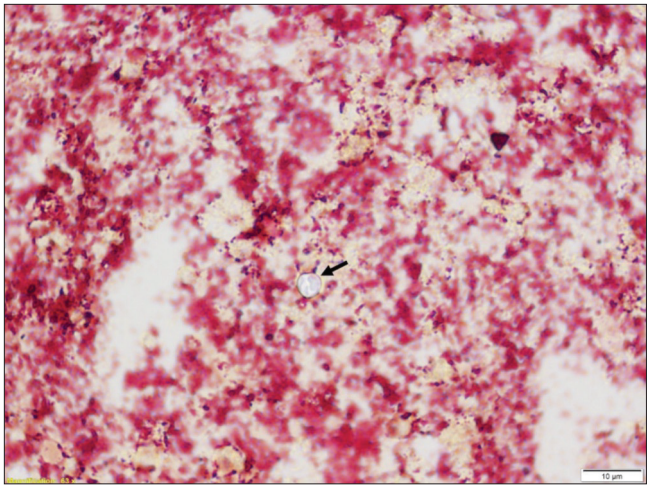

Fig 1. *Cryptosporidium* spp. oocyst that determined by the carbol-fuchsin staining method was shown with black arrow

amplify the SSU rRNA gene region of *Cryptosporidium*. 18SiCF2/18SiCR2 and 18SiCF1/18SiCR1 primer pairs were used for the first and second PCR amplification, respectively [24]. PCR reactions were performed in a total volume of 20 µL, consisted of a commercial master mix (Phusion High-Fidelity PCR Master Mix 5X, Thermo Scientific, Waltham, MA, USA), 0.5 µM each primer and 10-30 ng of gDNA. For the second PCR, 1 µL of the first PCR's product was used as template. An automated thermocycler (Applied Biosystems, Thermo Scientific, Waltham, MA, USA) were used in the PCR analyses and the amplification conditions (both first and second PCR amplifications) included a pre-denaturation at 98°C for 30 s, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 61.8°C for 20 s, and extension at 72°C for 45 s. The final extension step was carried out at 72°C for 10 min. Ultra-pure grade water and gDNA of *C. parvum* were used as the negative and positive controls in PCR assays, respectively. PCR amplicons (20 µL) were analyzed in 1.5% agarose gel, stained with SafeView™ (Applied Biological Materials, Richmond, BC, Canada), and visualized (Quantum CX5, Vilber Lourmat, France). Approximately 587 bp fragment of SSU rRNA gene region of *Cryptosporidium* isolate was amplified by nested PCR analysis and visualized on agarose gel *(Fig. 2-A)*. After that, the PCR product was purified (High Pure PCR Product Purification Kit, Roche, Germany) from gel to increase sequence quality. Purified product (5 µl) was re-analyzed in agarose gel to check the purification result *(Fig. 2-B)* and sequenced in both directions (Macrogen, Amsterdam, The Netherlands) using the nested PCR primers.

The obtained sequences were assembled and edited in Geneious Prime 2020.0.3 *(https://www.geneious.com)* by evaluating the quality value of sequence chromatograms. The forward and reverse sequence electropherograms were also visually checked for the presence of the double nucleotide peaks that might show possible mixed infections. The SSU rRNA gene consensus sequence (560 bp) was

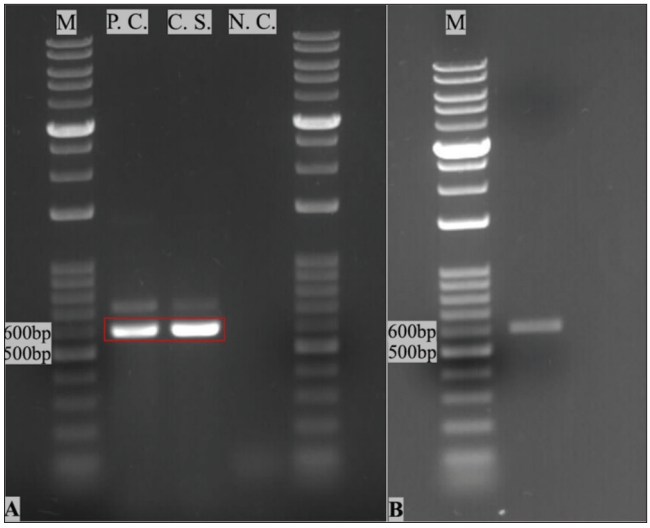

Fig 2. a) Gel electrophoresis image of nested PCR analyzes performed with primers 18SiCF1-18SiCR1 (SSU rRNA gene region). P.C.: Positive control, C.S.: Cat sample, N.C.: Negative control **b)** Gel electrophoresis image of purified amplicon for sequencing

successfully obtained from *Cryptosporidium* isolate. There was no double peak indicative of mixed infections. A BLASTn search was conducted using the final nucleotide sequences to make species-based identification and create a data set. The sequence and BLASTn analysis of the SSU rRNA gene region confirmed the microscopic examination result and *Cryptosporidium* isolate was molecularly assigned as *C. felis*. The nucleotide sequence of the SSU rRNA gene region of *C. felis* was deposited in GenBank with ANK_1 isolate name (accession number: MN394123).

For the phylogenetic analyses, the sequence data set was created by considering BLASTn results and using the available *Cryptosporidium* sequences (54 *C. felis* out of 61 *Cryptosporidium* isolates) from cats in GenBank. In created data set, trimmed alignment was 560 bp in length. The data set was tested with MEGA X model test to determine the most suitable DNA model according to the correct Akaike's Information Criterion (AIC). Phylogenetic analysis was conducted using the maximum-likelihood (ML) analysis based on GTR+G (the General Time Reversible + Gamma distributed) model in MEGA X [25] with 1000 bootstrap replicates. Genetic distances were determined using the Kimura two-parameter model [26] in MEGA X [25]. ANK_1 isolate showed a range from 99.1-100% identity with the most of *C. felis* sequences available in the GenBank *(Fig. 3)*. However, it exhibited 1.5%, 2%, 2.8%, 3.3%, and 4.3% high genetic distance with some of *C. felis* sequences with the accession numbers MG888051, KX174306, JQ312664, KX168415, and MG889862, respectively. The intraspecific genetic differences among all SSU rRNA sequences of *C. felis* isolates included in the data set were determined ranged from 0.0-6.9%. The ML tree based on the SSU rRNA gene region of *C. felis* revealed two main clades with the support of a 99% bootstrap value. Different *C. felis* haplotypes from various geographical regions were observed in the phylogenetic tree *(Fig. 3)*.

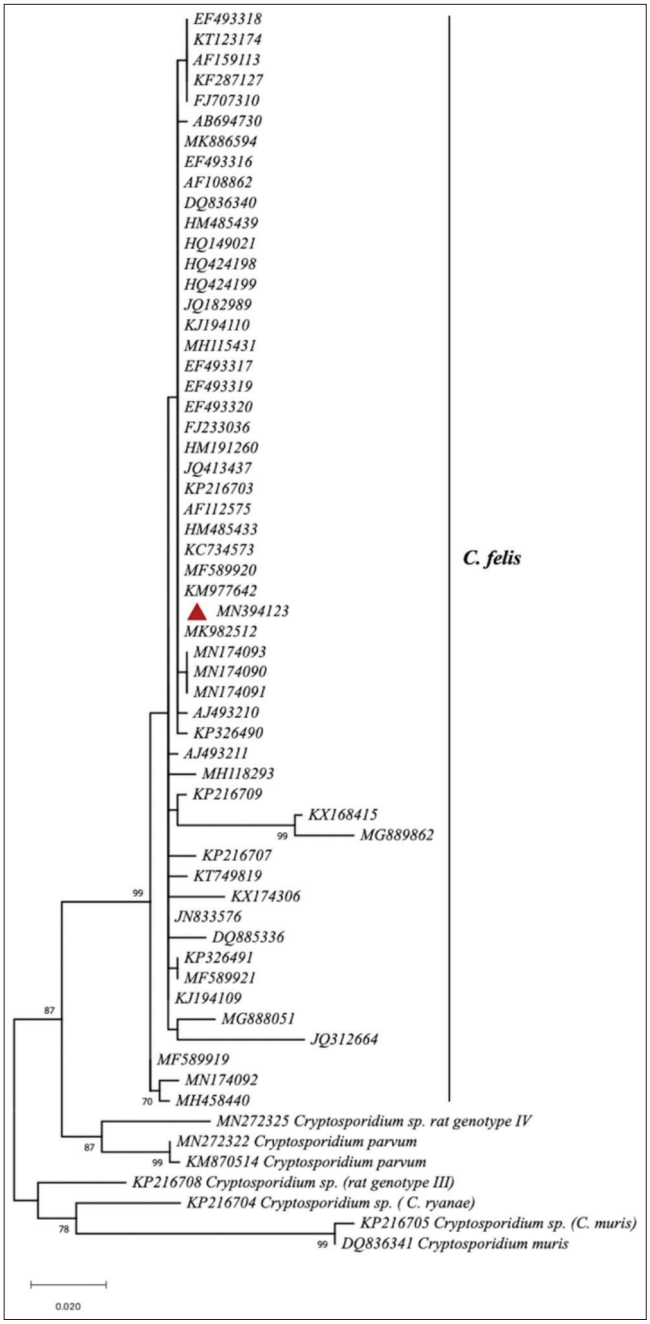

Fig 3. Phylogenetic relationships among *Cryptosporidium* isolates reported from cats in GenBank based on the SSU rRNA gene. Isolates that are included in data sets were given with GenBank accession numbers and species names. The isolate (ANK_1) found in this case report was shown with red character. Scale bar represents 0.020% substitutions per nucleotide position

DISCUSSION

Several risk factors such as breed, age, sex, and outdoor access have previously been analyzed for cryptosporidiosis in cats [27,28]. *Cryptosporidium* infection detected in this case might be related to the living area and age of the

cat. Because outdoor conditions could provide greater opportunity for young cats to exposure to infected hosts or contaminated soil/water. Some authors state that the infection risk with *Cryptosporidium* agents is higher in outdoor/stray cats than indoor/pet cats [27]. Besides cryptosporidiosis has been found more prevalent in young cats and this situation has been attributed with their immature immune system [28].

The prevalence of cryptosporidiosis in cats has been reported range from 0% to 29.4% in the world and these differences have been associated with diagnosis techniques used (reviewed by Lucio-Foster et al.[29]). The prevalence of *Cryptosporidium* spp. in cats has been reported from 1% to 13% in Turkey [19-21]. However, there is no data available regarding to molecular characterization of *Cryptosporidium* isolates in cats in Turkey. In this case, the SSU rRNA gene region of *Cryptosporidium* isolate found in a cat was successfully amplified and sequenced for the first time in Turkey. According to the BLASTn analysis, *Cryptosporidium* spp. isolate was molecularly assigned as *C. felis*, which is consistent with the reported dominance of this species in cats [4,6,30,31]. Similar to the findings of Ito et al.[31], ANK_1 isolate showed a range from 99.1% to 100% identity with most of *C. felis* sequences. However, high intraspecific genetic differences (0.0-6.9%) were determined among all SSU rRNA sequences of *C. felis* isolates included in the data set. Moreover, different *C. felis* haplotypes were observed in the phylogenetic tree. Considering high genetic variability, a suitable genetic marker may be needed to identify the subtypes family of the *C. felis* isolates.

Most of the human cases are associated with *C. hominis* and *C. parvum*. Besides these species, *C. felis, C. canis, C. meleagridis, C. cuniculus, C. ubiquitum,* and *C. viatorum* species are also responsible for human cryptosporidiosis [5,14]. Particularly, *C. felis* has been reported from many humans in different countries [32-35]. In a study, the identical *C. felis* SSU rRNA, HSP70, and COWP gene sequences were confirmed in both the cat and its immunocompetent owner in Sweden [33]. This result was an important step to molecularly confirm the zoonotic transmission of *C. felis* from cat to human. The authors highlighted that the cat could be the initial source of infection [33]. In another study on human cryptosporidiosis in the UK, contact with cats has been found as a significant risk factor for *C. felis* cases determined in humans [36].

In conclusion, we provided the first microscopic and molecular evidence of the occurrence of *C. felis* in a cat in Turkey. Considering the studies mentioned above, although *C. felis* has a relatively low zoonotic risk to public health compared with *C. parvum* [5,29], infected cats should not be neglected for zoonotic transmission due to close relationships between cats and humans. Especially, immunocompromised humans and children should be avoided from cats infected with *Cryptosporidium* spp. New studies with a large-scale sampling in a wide geographic area are necessary to determine prevalence, species, and genetic diversity of *Cryptosporidium* species in cats and to reveal potential risk factors for public health.

Statement of Author Contributions

NS designed the study. Parasitological examinations and molecular analyses were conducted by NS and ES. The manuscript was written by NS, and reviewed by ES and KY. All authors contributed to the improvement of discussion and reviewed the final manuscript not only for spelling and grammar but also for its intellectual content.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

REFERENCES

1. Xiao L, Fayer R, Ryan U, Upton SJ: *Cryptosporidium* taxonomy: Recent advances and implications for public health. *Clin Microbiol Rev,* 17 (1): 72-97, 2004. DOI: 10.1128/cmr.17.1.72-97.2004

2. Fayer R: Taxonomy and species delimitation in *Cryptosporidium*. *Exp Parasitol,* 124 (1): 90-97, 2010. DOI: 10.1016/j.exppara.2009.03.005

3. Feng Y, Ryan UM, Xiao L: Genetic diversity and population structure of *Cryptosporidium*. *Trends Parasitol,* 34 (11): 997-1011, 2018. DOI: 10.1016/j.pt.2018.07.009

4. Yang R, Ying JLJ, Monis P, Ryan U: Molecular characterisation of *Cryptosporidium* and *Giardia* in cats *(Felis catus)* in Western Australia. *Exp Parasitol,* 155, 13-18, 2015. DOI: 10.1016/j.exppara.2015.05.001

5. Ryan U, Fayer R, Xiao L: *Cryptosporidium* species in humans and animals: Current understanding and research needs. *Parasitology,* 141 (13): 1667-1685, 2014. DOI: 10.1017/S0031182014001085

6. Alves MEM, Martins FDC, Braunig P, Pivoto 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,* 117 (9): 3033-3038, 2018. DOI: 10.1007/s00436-018-5986-4

7. Tangtrongsup S, Scorza AV, Reif JS, Ballweber LR, Lappin MR, Salman MD: Seasonal distributions and other risk factors for *Giardia duodenalis* and *Cryptosporidium* spp. infections in dogs and cats in Chiang Mai, Thailand. *Prev Vet Med,* 174: 104820, 2020. DOI: 10.1016/j.prevetmed.2019.104820

8. 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,* 12 (1): 571, 2019. DOI: 10.1186/s13071-019-3822-z

9. Arai H, Fukuda Y, Hara T, Funakoshi Y, Kaneko S, Yoshida T, Asahi H, Kumada M, Kato K, Koyama T: Prevalence of *Cryptosporidium* infection among domestic cats in the Tokyo metropolitan district, Japan. *Jpn J Med Sci Biol,* 43 (1): 7-14, 1990. DOI: 10.7883/yoken1952.43.7

10. Mtambo MM, Nash AS, Blewett DA, Smith HV, Wright S: *Cryptosporidium* infection in cats: Prevalence of infection in domestic and feral cats in the Glasgow area. *Vet Rec,* 129 (23): 502-504, 1991. DOI: 10.1136/vr.129.23.502

11. Scorza V, Tangtrongsup S: Update on the diagnosis and management of *Cryptosporidium* spp infections in dogs and cats. *Top Companion Anim Med,* 25 (3): 163-169, 2010. DOI: 10.1053/j.tcam.2010.07.007

12. Khurana S, Chaudhary P: Laboratory diagnosis of cryptosporidiosis. *Trop Parasitol,* 8 (1): 2-7, 2018. DOI: 10.4103/tp.TP_34_17

13. Xiao L, Fayer R: Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission.

Int J Parasitol, 38 (11): 1239-1255, 2008. DOI: 10.1016/j.ijpara.2008.03.006

14. Xiao L: Molecular epidemiology of cryptosporidiosis: An update. *Exp Parasitol,* 124 (1): 80-89, 2010. DOI: 10.1016/j.exppara.2009.03.018

15. Arslan MÖ, İtik Ekinci A: Kars yöresinde sığırlarda *Cryptosporidium parvum* subtiplerinin belirlenmesi. *Kafkas Univ Vet Fak Derg,* 18 (Suppl. A): A221-A226, 2012. DOI: 10.9775/kvfd.2012.6565

16. Taylan-Ozkan A, Yasa-Duru S, Usluca S, Lysen C, Ye J, Roellig DM, Feng Y, Xiao L: *Cryptosporidium* species and *Cryptosporidium parvum* subtypes in dairy calves and goat kids reared under traditional farming systems in Turkey. *Exp Parasitol,* 170, 16-20, 2016. DOI: 10.1016/j.exppara.2016.06.014

17. Yildirim A, Adanir R, Inci A, Yukari BA, Duzlu O, Onder Z, Ciloglu A, Simsek E: Prevalence and genotyping of bovine *Cryptosporidium* species in the Mediterranean and Central Anatolia Region of Turkey. *Comp Immunol Microbiol Infect Dis,* 69: 101425, 2020. DOI: 10.1016/j.cimid.2020.101425

18. Yılmazer N, Küçük ŞK, Akyıldız G, Gargılı A, Kar S: Cryptosporidiosis in humans with reference to the first case of *Cryptosporidium hominis* infection in Turkey. *Med Bull Haseki,* 55: 194-198, 2017. DOI: 10.4274/haseki.47966

19. Korkmaz UF, Gökpınar S, Yıldız K: Kedilerde bağırsak parazitlerinin yaygınlığı ve halk sağlığı bakımından önemi. *Turkiye Parazitol Derg,* 40, 194-198, 2016. DOI: 10.5152/tpd.2016.4841

20. Göz Y, Yüksek N, Altug N, Ceylan E, Deger S: Prevalence of *Cryptosporidium* infection in Van cats. *Indian Vet J,* 82 (9): 995-996, 2005.

21. Kilinç ÖO, Yilmaz AB, Göz Y, Özkan C, Denizhan V: Determination of *Cryptosporidium* spp. in Van cats by nested PCR. *Med Weter,* 74 (7): 456-459, 2018. DOI: 10.21521/mw.6008

22. Purina: Fecal scoring chart 2016. https://www.proplanveterinarydiets.ca/wp-content/uploads/2016/04/PPPVD-Fecal-Scoring-Chart-EN-FINAL.pdf; *Accessed:* 12.02.2020.

23. Heine J: A simple technic for the demonstration of cryptosporidia in feces. *Zentralbl Veterinarmed B,* 29 (4): 324-327, 1982.

24. Ryan U, Xiao L, Read C, Zhou L, Lal AA, Pavlasek I: Identification of novel *Cryptosporidium* genotypes from the Czech Republic. *Appl Environ Microbiol,* 69 (7): 4302-4307, 2003. DOI: 10.1128/aem.69.7.4302-4307.2003

25. Kumar S, Stecher G, Li M, Knyaz C, Tamura K: MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol,* 35 (6): 1547-1549, 2018. DOI: 10.1093/molbev/msy096

26. Kimura M: A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol,* 16 (2): 111-120, 1980. DOI: 10.1007/BF01731581

27. McReynolds CA, Lappin MR, Ungar B, McReynolds LM, Bruns C, Spilker MM, Thrall MA, Reif JS: Regional seroprevalence of *Cryptosporidium parvum*-specific IgG of cats in the United States. *Vet Parasitol,* 80 (3): 187-195, 1999. DOI: 10.1016/s0304-4017(98)00219-2

28. Rambozzi L, Menzano A, Mannelli A, Romano S, Isaia MC: Prevalence of cryptosporidian infection in cats in Turin and analysis of risk factors. *J Feline Med Surg,* 9 (5): 392-396, 2007. DOI: 10.1016/j.jfms.2007.03.005

29. Lucio-Forster A, Griffiths JK, Cama VA, Xiao L, Bowman DD: Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. *Trends Parasitol,* 26 (4): 174-179, 2010. DOI: 10.1016/j.pt.2010.01.004

30. Kváč M, Hofmannová L, Ortega Y, Holubová N, Horčičková M, Kicia M, Hlásková L, Květoňová D, Sak B, McEvoy J: Stray cats are more frequently infected with zoonotic protists than pet cats. *Folia Parasitol,* 64: 034, 2017. DOI: 10.14411/fp.2017.034

31. Ito Y, Itoh N, Kimura Y, Kanai K: Molecular detection and characterization of *Cryptosporidium* spp. among breeding cattery cats in Japan. *Parasitol Res,* 115 (5): 2121-2123, 2016. DOI: 10.1007/s00436-016-4984-7

32. Bednarska M, Jankowska I, Pawelas A, Piwczynska K, Bajer A, Wolska-Kusnierz B, Wielopolska M, Welc-Faleciak R: Prevalence of *Cryptosporidium*, *Blastocystis*, and other opportunistic infections in patients with primary and acquired immunodeficiency. *Parasitol Res,* 117 (9): 2869-2879, 2018. DOI: 10.1007/s00436-018-5976-6

33. Beser J, Toresson L, Eitrem R, Troell K, Winiecka-Krusnell J, Lebbad M: Possible zoonotic transmission of *Cryptosporidium felis* in a household. *Infect Ecol Epidemiol,* 5 (1): 28463, 2015. DOI: 10.3402/iee.v5.28463

34. Raccurt CP: [Human *Cryptosporidium felis* infection in the world]. *Parasite,* 14 (1): 15-20, 2007. DOI: 10.1051/parasite/2007141015

35. Ebner J, Koehler AV, Robertson G, Bradbury RS, Jex AR, Haydon SR, Stevens MA, Norton R, Joachim A, Gasser RB: Genetic analysis of *Giardia* and *Cryptosporidium* from people in Northern Australia using PCR-based tools. *Infect Genet Evol,* 36, 389-395, 2015. DOI: 10.1016/j.meegid.2015.08.034

36. Elwin K, Hadfield SJ, Robinson G, Chalmers RM: The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000-2008. *Epidemiol Infect,* 140 (4): 673-683, 2012. DOI: 10.1017/S0950268811000860