Tehran University of Medical Sciences Publication
http://tums.ac.ir

Iran J Parasitol

Open access Journal at
http://ijpa.tums.ac.ir

Iranian Society of Parasitology
http://isp.tums.ac.ir

Original Article

The Role of Some Free-Ranging Animals in the Transmission of Multi-Host Species of *Cryptosporidium* Spp.

Mehran Mirzaghavami, *Javid Sadraei, Majid Pirestani, Saeed Bahadory

Department of Parasitology and Entomology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Received 12 Jun 2022
Accepted 08 Aug 2022

Keywords:
Cryptosporidium;
Mutli-host species;
Free-ranging;
Transmission

***Correspondence**
Email:
sadraeij@modares.ac.ir

Abstract

Background: We aimed to characterize *Cryptosporidium* spp. in rats, cats, pigeons, and crows.

Methods: Fifty-five animal origin *Cryptosporidium* spp. genome were identified, genotyped and confirmed by nested PCR and of RFLP-PCR analysis as well as sequenced based on 18s rRNA and *gp60* genes in Tehran (2012-2019). Finally, the phylogenetic analysis was performed by MEGA software (version 7).

Results: By the molecular method, *Cryptosporidium* spp. were detected in 24 (15.2%), 15 (15%), 2 (2%) and 13 (13%) cases of wild rats, cat, pigeon, and crow, respectively. Among the identified species by the RFLP pattern, most isolates were identified as *C. parvum* (24/157) 17.8% in rats, (15/100) 15% in cats, (13/100) 13%in crew and (2/100) 2% in pigeons; and the rest of the cases were *C. muris* and *C. felis*. The results of sequencing did not prove the existence of *C. parvum*, *C. felis*, *C. muris*, and rat genotype. Subtyping of *C. parvum* was indicated that the dominant subtype family belongs to the IId family and the subtype A20G1 was the most common subtype detected in all hosts while A19G1 was detected in one isolate of cat and pigeon.

Conclusion: Free-ranging animals are infected by species/subtype of *Cryptosporidium*, which can infect humans. This shows by itself the hygienic importance of the free-ranging animals in urban ecosystems. In the transmission of human cryptosporidiosis, the multi-host *Cryptosporidium* species such as *C. parvum*, *C. felis*, and *C. muris* can be transferred potentially from these animals to humans.

Introduction

Cryptosporidium spp. is a prevailing parasite of a vast range of animals including wildlife, companion animals, livestock, and humans (1). Clinical signs of cryptosporidiosis can be varied from self-limiting diarrhea and other gastrointestinal disorders to a chronically deathful infection in immunocompromised persons (2). Zoonotic and anthroponotic transmission routes have been identified for *Cryptosporidium* spp. and calves are considered as the main source in the zoonotic transmission cycle (3). The role of cattle in the zoonotic transmission of *C. parvum* is reported, but the role of the other animals is less clear (4).

Molecular studies on *Cryptosporidium* spp. in free-ranging animals in west Asia are lesser than those conducted in developed countries. *Cryptosporidium* oocysts can be detected everywhere in the natural surroundings which are contaminated by animal droppings or human wastes (5). They can contaminate soil, food, and water sources such as lakes, rivers, streams, and even swimming pools.

Up to now, limited molecular data are available for *Cryptosporidium* species in free-range animals in Iran. We aimed to characterize *Cryptosporidium* spp. in rat, cat, pigeon, and crow in Tehran capital of Iran.

Materials and Methods

Study area and sample collection

This study was conducted in Tehran, capital of Iran between 2012 and 2019. A total number of 457 fecal samples were randomly collected from rats (N=157), cats (N=100), pigeons (N=100), and crows (N=100) from different regions of the city during the course study from 2012 to 2019 ~~2016~~. All samples were preserved in 2.5% potassium dichromate (K2Cr2O7).

DNA extraction and Cryptosporidium species differentiation

DNA extraction was performed by the modified CTAB method (6). The 18S rRNA gene was amplified according to the nested PCR protocol described by Silva et al (7). The first and second PCR amplicons were about 773 bp and 611 bp, respectively. For species differentiation, according to enzymatic pattern with insilico digestion was determined species of *Cryptosporidium* by *Ssp*I, *Bfa*I, and *Dde*I restriction enzymes (Table 1).

Table 1: The RFLP patterns of *Cryptosporidium* spp., which is expected to isolate from the hosts in this study. Each color is a RFLP pattern. Similar colors represent the same pattern

Parasite	***BfaI***	***SspI***	***DdeI***
C. parvum	560	254-206-108	367-156-68
C. muris	364-195	385-203	225-208-156
C. canis	316-241	254-207-105	362-156-68
C. felis	582	391-206	387-156-68
C. ryanae	357-195	254-224-103	425-156
C. baileyi	360-195	330-254	428-156
C. meleagridis	561	254-206-108	366-156-68
C. ubiquitum	309-148-106	373-208	368-156-68

Subtyping of C. parvum

Isolates of *C. parvum* were subtyped using a 450-bp fragment *GP60* gene, which amplified by a nested PCR protocol (8). Amplified DNA fragments were purified and subjected to bidirectional sequencing.

Sequencing and Phylogenetic analysis

To identify the species/genotypes and confirmation of PCR-RFLP analysis, bidirectional sequencing was performed on the second PCR amplicons of 18S rRNA gene. The sequences were aligned with the available sequences of *Cryptosporidium* spp. in GenBank (www.ncbi.nlm.nih.gov/blast). The phylogenetic analysis was performed by MEGA software version 7. The phylogenetic trees were generated using the maximum likelihood method based on Kimura's two-parameter model with bootstrap values obtained from 1000 replicates.

Nucleotide sequence and accession numbers

The nucleotide sequences obtained in this study were submitted to GenBank under accession numbers (KX537648-KX537684) and (KP883285-KP883294).

Results

Prevalence and Cryptosporidium species identification

The numbers of positive isolates for each animal and the *Cryptosporidium* species/genotypes determined by 18s rRNA gene are summarized in Table 2.

Table 2: Number of positive samples examined for *Cryptosporidium* for each host and the *Cryptosporidium* species/genotypes/subtypes determined by PCR analysis of the SSU rRNA and GP60 genes

Host	*No. samples*	*Positive/ tested by 18S rRNA (%)*	*Species/genotype (%)*	*accession no.*	*Subtype family IId (%)*	*accession no.*
Rattus rattus	157	24 (17.8)	*C. parvum* (70.8) *C. muris* (16.7) Rat genotype (8.3) Mixed (4.2)	*C. parvum* (KP883285-288, KP883290-291 KP883293-294) *C. muris* (KX537656) Rat genotype (KP883289, KP883292)	A20G1 (100)	A20G1 (KX537657-668)
Felis catus	100	15 (15)	*C. parvum* (53.3) *C. felis* (33.3) Mixed (13.4)	*C. parvum* (KX537648, KX537653-654) *C. felis* (KX537655)	A20G1 (75) A19G1 (25)	A20G1 (KX537669-670) A19G1 (KX537672)
Corvus cornix	100	13 (13)	*C. parvum* (100)	*C. parvum* (KX537649-651)	A20G1 (100)	A20G1 (KX537657-684)
Columba livia	100	2 (2)	*C. parvum* (100)	*C. parvum* (KX537650)	A20G1 (50) A19G1 (50)	A20G1 (KX537674) A19G1 (KX537673)

The prevalence of *Cryptosporidium* spp. infections detected by 18S rRNA gene were (24/157) 17.8% in rats, (15/100) 15% in cats, (13/100) 13% in crew and (2/100) 2% in pigeons.

The sizes of fragments belonging to the majority of samples were identical to the expected RFLP patterns. The RFLP pattern of digested amplicons with *Ssp*I, *Fsp*BI (*BfaI*), and *HpyF3*I (*DdeI*) showed that 17 rat isolates had

a restriction pattern similar to *C. parvum*, 4 isolates similar to *C. muris*, 1 isolate mixed pattern and 2 isolates with the unknown pattern. Among 15 positive samples from cats 8 isolates had a restriction pattern with *C. parvum*, 5 isolate with *C. felis* and 2 isolates had mixed pattern. The RFLP derived from 13 crow and 2 pigeon isolates demonstrated an identical RFLP pattern with *C. parvum* (Fig. 1).

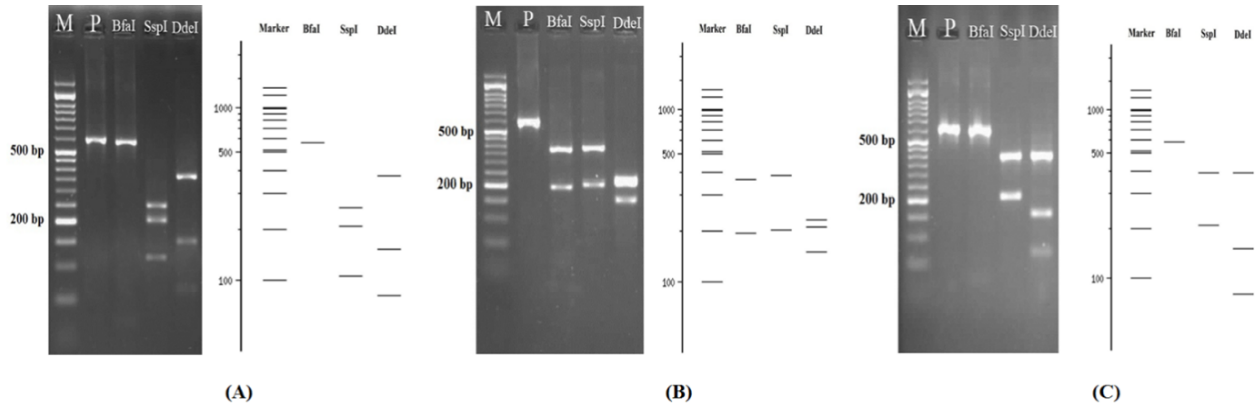

Fig. 1: The RFLP patterns of *Cryptosporidium* spp. (A) *C. parvum*, (B) *C. muris*, (C) *C. felis*

18S rRNA gene sequencing and phylogenetic analysis

The sequence of rat isolates showed 99-100% homology with *C. parvum* (17 isolates), four isolates with *C. muris* and, two isolates (unknown RFLP pattern) with rat genotype. The sequences of cat isolates showed 100% homology with *C. parvum* (8 isolates) one isolate identified 100% similar to *C. felis*. All sequences of crow and pigeon isolates showed 99-100% homology with *C. parvum*. In the phylogenetic analysis, the species isolated in this study grouped with previously described species (Fig. 2). The evolutionary history was concluded using the Maximum Likelihood method based on the Kimura 2-parameter model. The highest log likelihood (-2458.1429) is demonstrated in the tree. Primary tree (s) for the heuristic search were taken automatically using Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3618)). The rate variation model allowed for some sites to be evolutionarily invariable (+I), 34.3173% sites. Two samples isolated from the rat, whose RFLP patterns were unknown, were clustered with rat genotype in the phylogenetic analysis. The *Cryptosporidium* isolates from rats and cats grouped with intestinal species (*parvum*) and gastric species (*felis, muris*, and rat genotype).

GP60 gene sequencing and phylogenetic analysis

The *gp60* amplicons of twenty-eight *C. parvum* isolates were sequenced (Table 2). Sequence analysis revealed that the IId family was significantly dominant in this study. All IId subtype family detected from rats and crows belonged to the IId20AG1 subtype (100%), while IId subtype family detected from cats (3/4) and pigeons (1/2) belonged to the IId20AG1 and the rest subtypes from them were classified as IId19AG1 subtype (25%) and (50%), respectively. The evolutionary history was inferred by using the Maximum Likelihood method based on the Ki-

mura 2-parameter model (Fig. 2). The relationship of subtyped isolates and reference sequences of *C. parvum* approved the presence of the IId subtype family in isolates of free-ranging animals. These isolates were clustered with the reference sequence of *C. parvum* subtypes IId (A13G1, A14G1, A15G1, A16G1, A17G1, A18G1, A19G1, A20G1, A21G1, A22G1, A23G1, A24G1, A25G1, A26G1, and A27G1) with high internode value (i.e., 99) (Fig. 3). The worldwide distribution of *C. parvum* subtype families was presented in Fig 4.

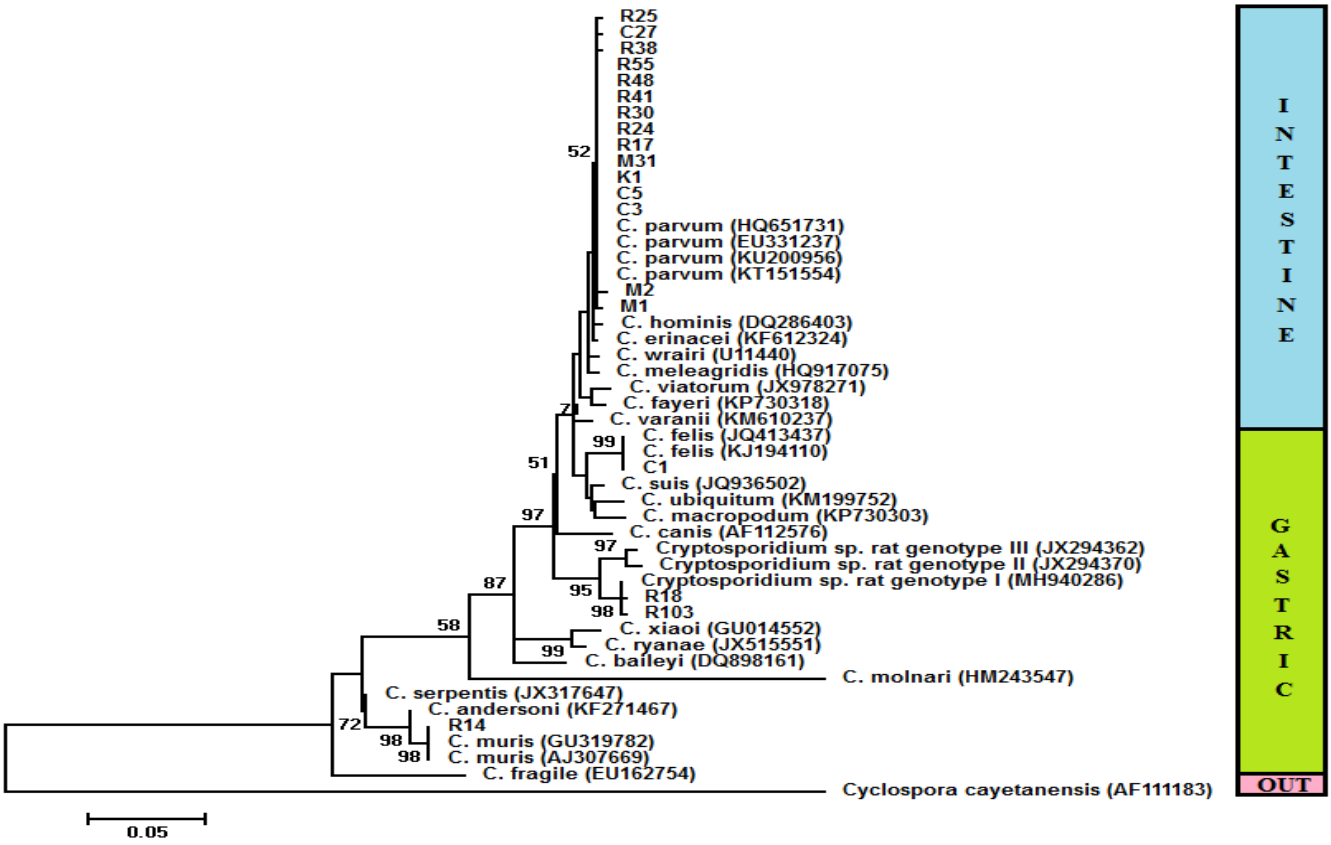

Fig. 2: Phylogenetic tree constructed from sequencing (18S) the *Cryptosporidium* isolates from free-ranging animals in Tehran. The sequences obtained from GenBank and used for a comparison with the sequences obtained in the present study included intestinal and gastric species of *Cryptosporidium*. Bootstrap values over 50% from 1000 replicates are indicated at the left of the node

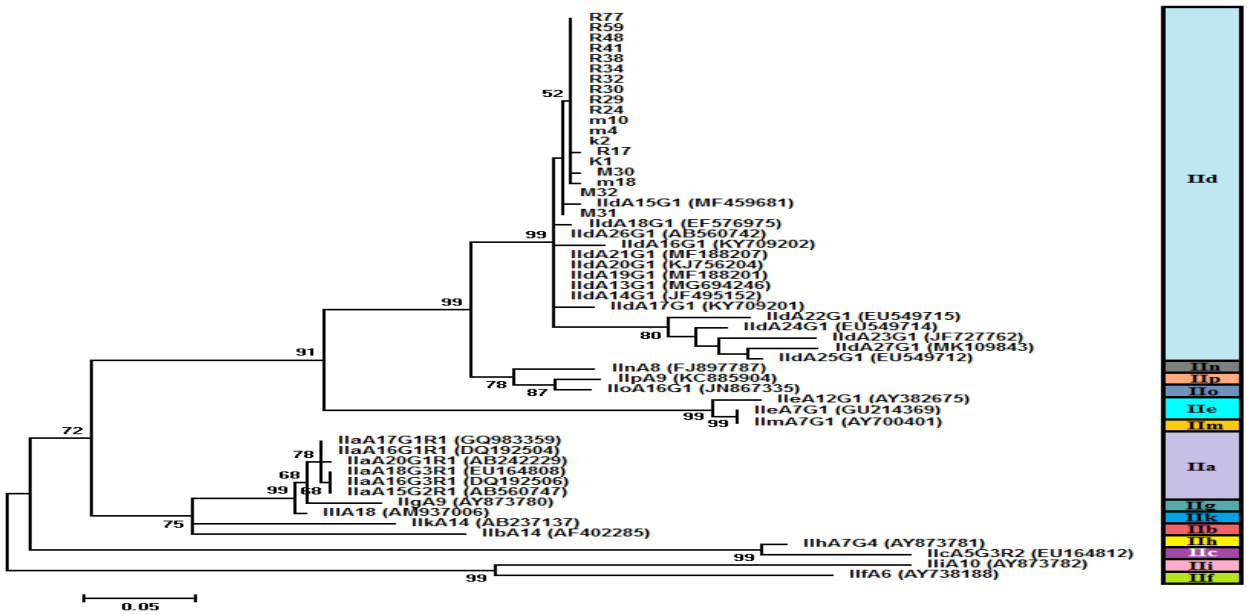

Fig. 3: Phylogenetic analysis of gp60 sequence data representing *C. parvum* from free-ranging animals using the Maximum Likelihood method based on the Kimura 2-parameter model. Sequences from the present study as well as reference sequences representing *C. parvum* subtypes (acquired from GenBank) are indicated. Bootstrap values over 50% from 1000 replicates are indicated at the left of the node

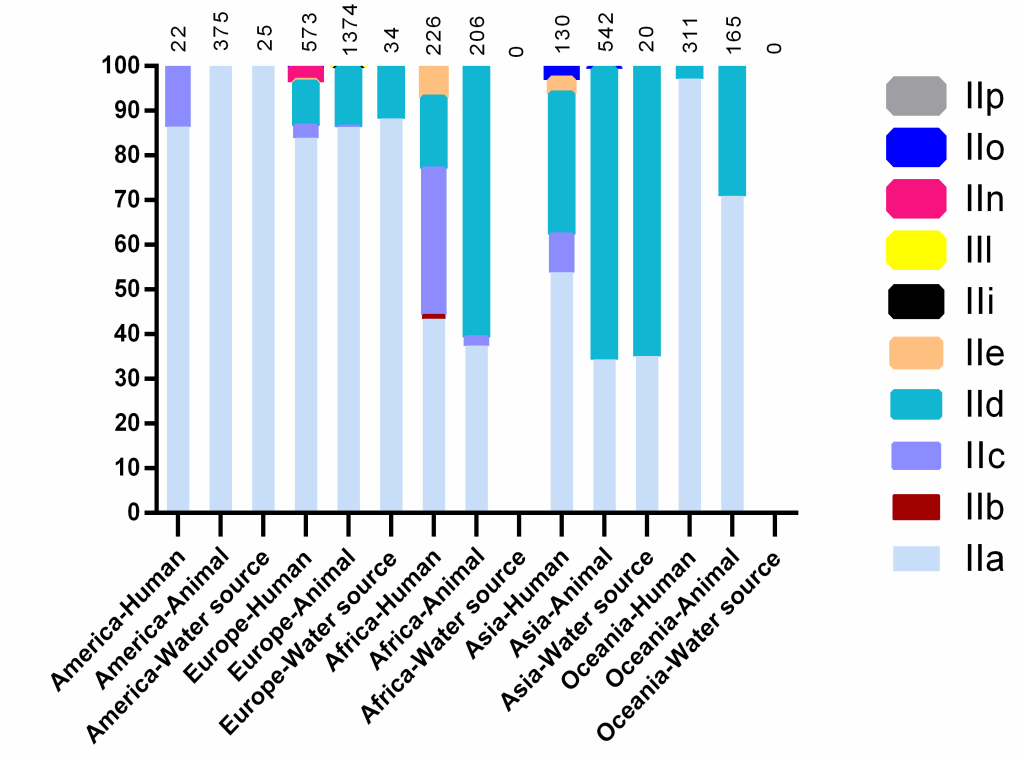

Fig. 4: Worldwide distribution of *C. parvum* subtype families. Numbers above bars represent numbers of isolates. Data are based on studies published between 2013 and March 2019

Discussion

The ubiquities *Cryptosporidium* oocysts can contaminate soil, food, and water sources (9). Previous studies about the molecular features of *Cryptosporidium* have been performed in Iran (10, 11); few data are available in free-range animals. For the first time in Iran, *C. muris* was detected in our study. The prevalence rates of *Cryptosporidium* spp. in rodents was reported in the range from 5.0% to 39.2% (10). In Tehran, 27% (21/77) wild rats were positive to *C. parvum* (11), whereas 17.8 % of the rats in our study were positive to *Cryptosporidium* spp. Thus far, rat genotype of *Cryptosporidium* and *C. muris* were not reported from the animal in Iran, but *C. muris* was detected in river water samples (12). The *Cryptosporidium* rat genotype was previously identified in two percent of laboratory mice and rats, 6.3% of *Rattus norvegicus*, and 9.1% of *Rattus tanezumi* in China (13). Contrary to studies conducted in Australia only rat genotype (14) have been detected, *C. parvum* and *muris* were identified in rats of China (15) which is consistent with our findings. The presence of multi-host *Cryptosporidium* species revealed that rats are one of the main sources of these parasites in the urban ecosystem. Urban communities have created suitable habitats for rats, therefore, close contact between rats and people in cities potentially provides transmission of zoonotic species of *cryptosporidium* (16).

The prevalence of *Cryptosporidium* spp. in cats was determined as 15% in our study. Among the positive isolate, *C. parvum*, *C. felis*, and mixed infection (*C. felis* and *muris*) were characterized. Some studies from elsewhere have confirmed the presence of *Cryptosporidium* in cats with prevalence ranging from 1 to 25% (17-19). Similar to our results, there are various reports in which detected *Cryptosporidium* species in cats. In Germany, *Cryptosporidium* spp. detected in one cat isolate with high identity values to *C. parvum* (20). Scrapings of duodenal and ileal mucosa and fecal specimens of cats from Colombia screened by PCR showed that six isolates (13%) positive for *Cryptosporidium* (5 isolates *C. felis* and one *C. muris*) (21). As in the present study, mixed *C. muris* and *C. felis* infection in a cat gastrointestinal tract reported from Australia (22) whereas, in another study, these species were separately isolated from the cat (22, 23). Koompapong et al. have genetically identified *Cryptosporidium* oocyst in 2.5% cat isolate as *C. felis* (24). Molecular analysis based on 18s rRNA gene revealed that one cat (3.8%) co-infected by *C. felis* and *C. parvum* in China (25). In addition, *C. muris* has been identified in cats (21, 26, 27). Characterization of zoonotic species of *Cryptosporidium* (*C. parvum*) in cats was shown they could be counted as potential reservoirs for human infections (28). Based on present knowledge, *C. felis* and *C. muris* have low public health significance in the general population. However, immunocompromised persons are at risk of serious cryptosporidiosis (29, 30).

Many varieties of free-ranging, captive, and domestic bird species can defecate human virulent agents in their fecal droppings (31). More than 30 species of birds are infected by *Cryptosporidium* spp. in many countries (32). They are used as a source of food, hobby, symbol, and experimental aims (33). In this study, the feces of two percent of pigeons and 13% of crows were infected by *C. parvum*, while the prevalence of *C. parvum* oocysts varies considerably in birds as high as 90% (34). On the other hand, unlike former studies that have performed using the microscopic method (35), this study showed the molecular aspects of *Cryptosporidium* in pigeon for the first time in Tehran. In Brazil, *C. parvum* was detected in seven percent of carrier pigeons (36). The prevalence rate of *Cryptosporidium* infection of Corvidae indicates the fluctuation of this infection from 0 to 33.96% (37).

GP60 gene analysis revealed 15 subtype families for (Cryptosporidium) *C. parvum* at least including IIa-IIi, IIk-IIp (novel subtype)

(38-40), while, ten subtype families were reported in *C. hominis* (Ia, Ib, Id, Ie, If, Ig, Ih, Ii, Ij, and Ik (novel subtype) (39). In wildlife, subtype families of *Cryptosporidium* spp. were characterized such as *C. hominis* (Ia, Ib, Id, Ie, If, Ii, and Ik), *C. parvum* (IIa, IIc, IId, IIo, and IIp), *C. cuniculus* (Va and Vb), *C. ubiquitum* (XIIa to f), *C. erinacei* (XIIIa), *C. fayeri* (IVa to f), opossum genotype I (XIa), *C. meleagridis* (IIIb, IIIe, and IIIg), *C. tyzerri* (IXa and IXb), *C. wrairi* (VIIa), chipmunk genotype I (XIVa), ferret genotype (VIIIa), horse genotype (VIa and VIb) and mink genotype (Xa) (40).

Formerly, both IIa (64%) and IId families of *C. parvum* (36%) were reported in cattle and humans in Iran. However, the subtype family IIa and IId predominated in cattle and humans, respectively. In children, five subtypes were identified in the IId subtype family (A15G1, A18G1, A20G1a, A21G1a, and A26G1) with the dominancy of A20G1a (41). In Tehran, all subtypes of *C. parvum* isolates belonged to families IIa (35%) and IId (65%) and the frequent subtype among the isolates of Iranian children was IIdA20G1 (42, 43).

The dominance of the subtype IIdA20G1 in this study is in accordance with the results of former studies in the countries of the Middle East region, which a IIa is dominant subtype (44). Subtype IIdA19G1 identified in 1 cat isolate and 1 pigeon isolate was previously identified from livestock in Sweden (45), China (46, 47), Hungary (48), Egypt (49), and Australia (39). This subtype was also common in lambs and goats in Spain (50) and equine hosts in China (51). In wildlife, the subtype IIdA19G1 was reported from golden takins, lemurs, chipmunks, hamsters, and hedgehog (52). The zoonotic nature of this subtype has been proven by detection in Spanish (53), Portuguese (54), and Ethiopian (55) individuals.

Detection of multi-host species of *Cryptosporidium* and common subtypes of *C. parvum* (such as IIdA20G1) in free-ranging animals in the present study, firmly suggests the potential role of these animals in the transmission of cryptosporidiosis. It is difficult to precisely identified sources and transmission dynamics of *Cryptosporidium* in the environment, for its omnipresent nature, however, zoonotic transmission is considered the main route in the transmission of cryptosporidiosis (56).

Conclusion

Considering the presence of rat, cat, pigeon, and crow animals in urban ecosystems it is probable that water resources, food, soil, animals, and humans are contaminated by these hosts. Therefore, these animals may probably be significant in terms of public health, and measures should be developed to reduce the transmission of (oo) cysts to humans, especially in susceptible persons. Increasing health awareness, screening, and reducing the risk of co-transmission between humans and animals in multi-host *Cryptosporidium* spp. seems necessary.

Acknowledgements

This study is result of Ph.D. thesis supported by Vice-Chancellor for Research of Tarbiat Modares University and Iranian National Sciences. This research was funded by the Office of President, Vice-Presidency for Science and Technology, Iran National Science Foundation (grant No.: 93012239).

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships.

References

1. Checkley W, White Jr AC, Jaganath D, et al. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. Lancet Infect Dis. 2015;15(1):85-94.
2. Vinayak S, Pawlowic MC, Sateriale A, et al. Genetic modification of the diarrheal pathogen

Cryptosporidium parvum. Nature. 2015;523(7561):477-480.
3. Taghipour A, Olfatifar M, Foroutan M, Bahadory S, Malih N, Norouzi M. Global prevalence of *Cryptosporidium* infection in rodents: A systematic review and meta-analysis. Prev Vet Med. 2020; 182:105119.
4. Ryan U, Fayer R, Xiao L. *Cryptosporidium* species in humans and animals: Current understanding and research needs. Parasitology. 2014;141(13):1667-1685.
5. Taghipour A, Olfatifar M, Bahadory S, et al . The global prevalence of *Cryptosporidium* infection in dogs: A systematic review and meta-analysis. Vet Parasitol. 2020; 281:109093.
6. Minas K, McEwan NR, Newbold CJ, Scott KP. Optimization of a high-throughput CTAB-based protocol for the extraction of QPCR-grade DNA from rumen fluid, plant and bacterial pure cultures. FEMS Microbiol Lett. 2011;325(2):162-169.
7. Silva SO, Richtzenhain LJ, Barros IN, et al. A new set of primers directed to *18s RRNA* gene for molecular identification of *Cryptosporidium* spp. And their performance in the detection and differentiation of oocysts shed by synanthropic rodents. Exp Parasitol. 2013;135(3):551-557.
8. Iqbal A, Lim YA, Surin J, Sim BL. High diversity of *Cryptosporidium* subgenotypes identified in Malaysian HIV/AIDS individuals targeting *gp60* gene. PLoS One. 2012;7(2):e31139.
9. Karanis P. *Cryptosporidium*: Waterborne and foodborne transmission and worldwide outbreaks. Euro-Mediterranean Conference for Environmental Integration. 2017:41-44
10. Foo C, Farrell J, Boxell A, Robertson I, Ryan UM. Novel *Cryptosporidium* genotype in wild Australian mice (*Mus domesticus*). Appl Environ Microbiol. 2007;73(23):7693-7696.
11. Bahrami F, Sadraei J, Frozandeh M. Molecular characterization of *Cryptosporidium* spp. In wild rats of Tehran, Iran using *18s rRNA* gene and PCR_RFLP method. Jundishapur Journal of Microbiology. 2012; 5(3):486-90.
12. Mahmoudi M, Nazemalhosseini-Mojarad E, Kazemi B, et al. *Cryptosporidium* genotypes and subtypes distribution in river water in Iran. J Water Health. 2015; 13(2):600-606.
13. Lv C, Zhang L, Wang R,et al. *Cryptosporidium spp*. In wild, laboratory, and pet rodents in China: Prevalence and molecular characterization. Appl Environ Microbiol. 2009; 75(24):7692-7699.
14. Paparini A, Jackson B, Ward S, Young S, Ryan UM. Multiple *Cryptosporidium* genotypes detected in wild black rats (*Rattus rattus*) from northern Australia. Exp Parasitol. 2012; 131(4):404-412.
15. Zhao Z, Wang R, Zhao W, et al. Genotyping and subtyping of *Giardia* and *Cryptosporidium* isolates from commensal rodents in china. Parasitology. 2015; 142(6):800-806.
16. Bradley CA, Altizer S. Urbanization and the ecology of wildlife diseases. Trends Ecol Evol. 2007;22(2):95-102.
17. da Silveira-Neto L, Inácio S, Oliveira L, Bresciani K. la cryptosporidiosis es una enfermedad subestimada en los gatos? Arch Med Vet. 2015;47:1-6.
18. Kváč M, Hofmannová L, Ortega Y, et al. Stray cats are more frequently infected with zoonotic protists than pet cats. Folia Parasitol (Praha). 2017; 64:034.
19. Lucio-Forster A, Griffiths JK, Cama VA, Xiao L, Bowman DD. Minimal zoonotic risk of cryptosporidiosis from pet dogs and cats. Trends Parasitol. 2010; 26(4):174-179.
20. Sotiriadou I, Pantchev N, Gassmann D, Karanis P. Molecular identification of *Giardia* and *Cryptosporidium* from dogs and cats. Parasite. 2013; 20:8.
21. Santín M, Trout JM, Vecino JAC, Dubey J, Fayer R. *Cryptosporidium*, *Giardia* and *Enterocytozoon bieneusi* in cats from Bogota (Colombia) and genotyping of isolates. Vet Parasitol. 2006; 141(3-4):334-339.
22. FitzGerald L, Bennett M, Ng J, et al. Morphological and molecular characterisation of a mixed *Cryptosporidium muris*/*Cryptosporidium felis* infection in a cat. Vet Parasitol. 2011;175(1-2):160-164.
23. Yang R, Ying JLJ, Monis P, Ryan U. Molecular characterisation of *Cryptosporidium* and *Giardia* in cats (felis catus) in western Australia. Exp Parasitol. 2015; 155:13-18.
24. Koompapong K, Mori H, Thammasont-hijarern N, et al. Molecular identification of *Cryptosporidium spp*. In seagulls, pigeons, dogs, and cats in Thailand. Parasite. 2014;21:52.

25. Li W, Li Y, Song M, et al. Prevalence and genetic characteristics of *Cryptosporidium*, *Enterocytozoon bieneusi* and *Giardia duodenalis* in cats and dogs in Heilongjiang province, china. Vet Parasitol. 2015;208(3-4):125-134.
26. Ehsan M, Akter M, Ahammed M, Ali M, Ahmed M. Prevalence and clinical importance of *Cryptosporidium* and *Giardia* in human and animals. Bangladesh Journal of Veterinary Medicine. 2016;14:109-122.
27. Pavlasek I, Ryan U. The first finding of a natural infection of *Cryptosporidium muris* in a cat. Vet Parasitol. 2007;144(3-4):349-352.
28. Ayinmode A, Obebe O, Falohun O. Molecular detection of *Cryptosporidium* species in street-sampled dog feces in Ibadan, Nigeria. Vet Parasitol Reg Stud Reports. 2018; 14:54-58.
29. Bowman DD, Lucio-Forster A. Cryptosporidiosis and giardiasis in dogs and cats: Veterinary and public health importance. Exp Parasitol. 2010;124(1):121-127.
30. Katsumata T, Hosea D, Ranuh IG, Uga S, Yanagi T, Kohno S. Possible *Cryptosporidium muris* infection in humans. Am J Trop Med Hyg. 2000;62(1):70-72.
31. Graczyk TK, Majewska AC, Schwab KJ. The role of birds in dissemination of human waterborne enteropathogens. Trends Parasitol. 2008;24:55-59.
32. Morgan UM, Monis PT, Xiao L,et al. Molecular and phylogenetic characterization of *Cryptosporidium* from birds. Int J Parasitol. 2001;31(3):289-296.
33. Cooper J. A veterinary approach to pigeons. J Small Anim Pract. 1984;25:505-516.
34. Wildi K, Haaf P, Reichlin T, et al. Uric acid for diagnosis and risk stratification in suspected myocardial infarction. Eur J Clin Invest. 2013;43(2):174-182.
35. Badparva E, Ezatpour B, Azami M, Badparva M. First report of birds infection by intestinal parasites in Khorramabad, west Iran. J Parasit Dis. 2015;39(4):720-724.
36. Oliveira BCM, Ferrari ED, da Cruz Panegossi MF, et al. First description of *Cryptosporidium parvum* in carrier pigeons (*Columba livia*). Vet Parasitol. 2017; 243:148-150.
37. Heuvelink A, Zwartkruis J, Van Heerwaarden C, Arends B, Stortelder V, De Boer E. Pathogenic bacteria and parasites in wildlife and surface water. Tijdschr Diergeneeskd. 2008;133(8):330-335.
38. Fayer R, Xiao L. *Cryptosporidium* and cryptosporidiosis. CRC press; 2007.
39. Zahedi A, Phasey J, Boland T, Ryan U. First report of *Cryptosporidium* species in farmed and wild buffalo from the northern territory, Australia. Parasitol Res. 2016;115(3):1349-1353.
40. Zahedi A, Paparini A, Jian F, Robertson I, Ryan U. Public health significance of zoonotic *Cryptosporidium* species in wildlife: Critical insights into better drinking water management. Int J Parasitol Parasites Wildl. 2016;5:88-109.
41. Nazemalhosseini-Mojarad E, Haghighi A, Taghipour N, et al. Subtype analysis of *Cryptosporidium parvum* and *Cryptosporidium hominis* isolates from humans and cattle in Iran. Vet Parasitol. 2011;179(1-3):250-252.
42. Taghipour N, Nazemalhosseini-Mojarad E, Haghighi A, et al. Molecular epidemiology of cryptosporidiosis in Iranian children, Tehran, Iran. Iran J Parasitol. 2011;6(4):41-45.
43. Sharbatkhori M, Mojarad EN, Taghipour N, Pagheh AS, Mesgarian F. Prevalence and genetic characterization of *Cryptosporidium spp.* In diarrheic children from Gonbad Kavoos city, Iran. Iran J Parasitol. 2015; 10(3):441-7.
44. Alyousefi N, Mahdy M, Lim YAL, Xiao L, Mahmud R. First molecular characterization of *Cryptosporidium* in Yemen. Parasitology. 2013; 140(6):729-734.
45. Silverlås C, Bosaeus-Reineck H, Näslund K, Björkman C. Is there a need for improved *Cryptosporidium* diagnostics in Swedish calves? Int J Parasitol. 2013;43(2):155-161.
46. Sun H, Zhuo X, Zhao X, et al. The heat shock protein 90 of *Toxoplasma gondii* is essential for invasion of host cells and tachyzoite growth. Parasite. 2017;24:22.
47. Wang R, Wang H, Sun Y, et al. Characteristics of *Cryptosporidium* transmission in preweaned dairy cattle in Henan, China. J Clin Microbiol. 2011; 49(3):1077-1082.
48. Plutzer J, Karanis P. Genotype and subtype analyses of *Cryptosporidium* isolates from cattle in Hungary. Vet Parasitol. 2007; 146(3-4):357-362.
49. Maizels RM. *Toxocara canis*: Molecular basis of immune recognition and evasion. Vet Parasitol. 2013;193(4):365-374.
50. Quílez J, Torres E, Chalmers RM, Hadfield SJ, Del Cacho E, Sánchez-Acedo C.

Cryptosporidium genotypes and subtypes in lambs and goat kids in Spain. Appl Environ Microbiol. 2008;74(19):6026-6031.

51. Jian F, Liu A, Wang R, et al. Common occurrence of *Cryptosporidium hominis* in horses and donkeys. Infect Genet Evol. 2016; 43:261-266.
52. Sangster L, Blake DP, Robinson G, et al. Detection and molecular characterisation of *Cryptosporidium parvum* in British European hedgehogs (*Erinaceus europaeus*). Vet Parasitol. 2016; 217:39-44.
53. de Lucio A, Merino FJ, Martínez-Ruiz R, Bailo B, Aguilera M, Fuentes I, Carmena D. Molecular genotyping and sub-genotyping of *Cryptosporidium* spp. Isolates from symptomatic individuals attending two major public hospitals in Madrid, Spain. Infect Genet Evol. 2016;37:49-56.
54. Alves M, Xiao L, Antunes F, Matos O. Distribution of *Cryptosporidium* subtypes in humans and domestic and wild ruminants in Portugal. Parasitol Res. 2006;99(3):287-292.
55. Coyle CM, Mahanty S, Zunt JR, et al. Neurocysticercosis: Neglected but not forgotten. PLoS Negl Trop Dis. 2012;6(5):e1500.
56. Xiao L. Molecular epidemiology of cryptosporidiosis: An update. Exp Parasitol. 2010;124(1):80-89.