Original Article

Respiratory and intestinal zoonotic cryptosporidiosis in symptomatic domestic pigeons (*Columba livia domestica*) in Tabriz, Iran

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Abstract

Background: Domestic pigeons (*Columba livia domestica*) are the oldest domesticated birds worldwide, harboring many zoonotic parasites and posing potential public health threats. **Aims:** To investigate cryptosporidiosis in domestic pigeons in Tabriz, Iran, 100 privately owned pigeons presenting weight loss and diarrhea were tested for *Cryptosporidium* spp. through parasitological, histopathological, and molecular tests. **Methods:** Modified Ziehl-Neelsen-stained fecal smears and histological sections of the trachea and small intestine were examined microscopically. Genomic DNA of fecal and tracheal specimens was examined by nested conventional PCR targeting *18S rDNA*, followed by Sanger sequencing of histopathology-confirmed samples and phylogenetic analyses. **Results:** All pigeons were positive at PCR in their feces and trachea. Oocysts similar to the size of *Cryptosporidium* species were observed in stained fecal smears of 62% of pigeons. At the histopathological examination, *Cryptosporidium*-organisms were observed on the apical epithelial surfaces of the small intestine in 84% and trachea in 78% of pigeons. In 23 pigeons, simultaneous tracheal and intestinal cryptosporidiosis was determined. The lesions in affected tracheas and small intestines included hyperemia, villous atrophy and fusion, dilatation of intestinal crypts, irregular epithelial hyperplasia, and sloughing. Diffused mixed inflammatory cell infiltration in the lamina propria was observed, with dominant lymphocytes, plasma cells, and lower numbers of heterophils. Consensus sequences of detected parasites revealed infection with *Cryptosporidium parvum* and *Cryptosporidium meleagridis*. **Conclusion:** Considering the high frequency of cryptosporidiosis reported here in symptomatic birds and that both identified *Cryptosporidium* species are zoonotic parasites, findings claim a public health risk assessment of this species of animals.

Key words: *Cryptosporidium*, Histopathology, Iran, Pigeon, Zoonosis

Introduction

Cryptosporidiosis is a protozoan parasitic disease of humans, various birds and other animal species (Ryan, 2010; Ryan *et al*., 2016). At least 49 *Cryptosporidium* spp. and more than 120 genotypes are currently identified (Prediger *et al*., 2021). In birds, six species, i.e., *C. baileyi*, *C. meleagridis*, *C. galli*, *C. avium*, *C. ornithophilus*, *C. proventriculi*, and several yet unnamed genotypes have been reported so far (Ryan *et al*., 2021). The mammal-related *Cryptosporidium* species such as *C. andersoni*, *C. hominis*, *C. muris*, *C. parvum*, and *C. canis* have also been identified in fecal specimens of birds (Sréter and Varga, 2000; Santín *et al*., 2004; Ng *et al*., 2006; Nakamura *et al*., 2009; Qi *et al*., 2011; Nakamura and Meireles, 2015; Helmy *et al*., 2017; Oliveira *et al*., 2017; Ferrari *et al*., 2018).

Cryptosporidium is an important pathogen in poultry that mainly causes respiratory and intestinal disease, leading to morbidity and mortality (Nakamura and Meireles, 2015). However, avian cryptosporidia are known to have multi-organ affinity. For instance, *C. baileyi* infects primarily the upper respiratory tract but also the bursa of Fabricius and cloaca, kidneys, and eyes of gallinaceous birds, leading to severe outbreaks and mortalities in game birds and poultry production units (Nakamura and Meireles, 2015). Among the *Cryptosporidium* species that mainly infect birds, *C. meleagridis* is reported in humans more than others (Feng *et al*., 2018). It affects primarily the ileum of

turkey poults and game birds and causes enteritis, diarrhea, and death (WOAH, 2022). Furthermore, there are known cases of human infection with *C. baileyi* (Ditrich *et al*., 1991; Kopacz *et al*., 2020). However, although birds usually get infected with host-adapted *Cryptosporidium* species, growing evidence suggests that birds act as mechanical transporters of zoonotic species such as *C. parvum* and *C. hominis* (Abreu-Acosta *et al*., 2009; Quah *et al*., 2011). Interestingly, *C. parvum* which is one of the most common *Cryptosporidium* species in humans worldwide, has been isolated from asymptomatic avian species such as chickens and turkeys (Xiao and Feng, 2008; McEvoy and Giddings, 2009; Helmy *et al.*, 2017; Shahbazi *et al.*, 2020) although association of *C. parvum* with catarrhal enteritis has been documented in the stone curlew (*Burhinus oedicnemus*) (Zylan *et al*., 2008). Accordingly, contamination of food and water supplies by bird droppings has been suggested as a possible transmission route of cryptosporidia to human populations (Zahedi *et al*., 2016).

Birds belonging to the genus *Columba*, especially the cosmopolitan rock domestic pigeons (*Columba livia domestica*), may pose public health concerns since they can potentially disseminate zoonotic pathogens and serve as reservoirs of several parasites (Lallo *et al*., 2012). Indeed, domestic pigeons are bred worldwide for meat, eggs, sporting competitions, or as pets (Harlin, 1994), and their close association with humans, animals, and other birds renders them a potential carrier and reservoir of zoonotic infections (Adang *et al*., 2008). Unfortunately, the molecular information on *Cryptosporidium* species infecting pigeons is scant, with only *C. hominis*, *C. parvum*, *C. meleagridis*, and *C. baileyi* are reported in fecal specimens of pigeons (Abreu-Acosta *et al*., 2009). More importantly, the above study is merely focused on the detection of the parasites in excretions and reports on pathologies associated with cryptosporidiosis in pigeons is limited (Rodriguez *et al*., 1997). In Iran, only few studies have addressed cryptosporidiosis in pigeons (Radfar *et al*., 2012; Mirzaghavami *et al*., 2016). None of these studies included the molecular identification of *Cryptosporidium* species. Pigeons are popular birds in Iran and have been kept as pets for entertainment, flying competitions, and production of meat and eggs since ancient times. Therefore, the present study was conducted to assess the frequency and pathological features of *Cryptosporidium* spp. infection in rock domestic pigeons with clinical signs including weight loss and diarrhea by histopathological and molecular examinations.

Materials and Methods

Ethical statement

All applicable international, national, and institutional guidelines for the care and use of animals were followed. Sampling was approved by the Research Ethics Committee of the University of Tabriz (ID: IR.TABRIZU.REC.1400.004).

Study area

This study was performed in Tabriz city, the capital of East-Azerbaijan Province, northwestern Iran (38.0792° N, 46.2887° E, 1351 m above sea level) (Ghiai *et al*., 2021) with a population of 1,643,960 inhabitants in 2022. It is located in the middle of the East Azerbaijan and the northeast part of Urmia Lake (Fig. 1). The city has a tropical and subtropical steppe climate (Köppen-Geiger classification: BSk) (Ghiai *et al*., 2021) with a yearly rainfall of ca. 360 mm.

Fig. 1: Map of Iran showing the location of the study area

Sample collection

From April to June 2021, a total of 100 domestic pigeons (rock pigeon, *Columba livia domestica*) were purchased from different locations in the city. As there was no information about the prevalence of *Cryptosporidium* spp. in this region, sampling was done based on a non-probability sampling method (i.e. convenience sampling). Bird owners reported clinical symptoms in pigeons, such as weight loss and diarrhea. After the decapitation of the pigeons with a sharp knife, a systematic necropsy was performed, and different organs and tissues were examined carefully for probable gross lesions such as vascular congestion and hemorrhage. Besides, the age and gender of the birds were recorded based on the owner's information, which later was confirmed at necropsy by observing the development and size of the sexual organs.

Fecal samples as well as tracheal (middle part) and small intestinal (ileum) tissue samples were then collected separately. Part of the fecal samples was smeared on glass slides, air-dried, and fixed with methanol for microscopic examinations. The rest of the fecal samples and a part of the tracheal specimens were stored at -20°C for molecular studies. Tissue samples of the small intestine and trachea were fixed in 10% neutral buffered formalin for histopathology.

Microscopic examinations

Thin fecal smears were stained with the modified Ziehl-Neelsen method (MZN) (Taylor *et al*., 2016) and examined using a light microscope. Briefly, the diameter of stained oocysts were measured using a bright-field microscope (Olympus, Japan) equipped with an Olympus

SPlan ×100 oil immersion lens, and an ocular micrometer carefully calibrated by a stage micrometer. Ten oocysts (n=10) in each stained smear were measured and the range was calculated.

Histopathological examinations

Tissue samples were fixed in neutral buffered formalin for at least 48 h. Two longitudinal sections of the intestine and one transverse section of the trachea were then processed routinely, dehydrated in ethanol, cleared and impregnated in xylol, embedded in paraffin, sectioned, and stained using hematoxylin-eosin (H&E). The tissue sections (n=300) were inspected by an ordinary light microscope (Olympus CH-30, Japan) and the presence of the parasite and probable histopathological lesions such as hyperemia, edema, hemorrhages, epithelial cell degeneration, cell or tissue necrosis, and inflammatory cell infiltration were examined.

DNA isolation and PCR assay

Fecal and tracheal tissue samples (ca. 200 mg) were thoroughly homogenized, using disposable plastic applicators; three freeze/thaw cycles were then performed, and finally, the resultant post-centrifugation supernatant (12,000×g at 4°C for 20 min) was used for DNA extraction. The genomic DNA (gDNA) was extracted, using a DNA extraction kit® (MBST, Tehran, Iran) based on the manufacturer's instructions. Quantitative and qualitative assessments were performed, using NanoDrop 2000™ (Thermo Scientific, Waltham, MA, USA) and 0.8% agarose gels. DNAs were tested for the presence of *Cryptosporidium* spp. by conventional nested PCRs targeting *18S rDNA*. Primer pairs SHP1/SHP2 (nest-1) (5´-ACC TAT CAG CTT TAG ACG GTA GGG TAT-3´ / 5´-TCT CAT AAG GTG CTG AAG GAG TAA GG-3´) and SHP3/SSU-R3 (nest-2) (5´-ACA GGG AGG TAG TGA CAA GAA ATA ACA-3´ / 5´-AAG GAG TAA GGA ACA ACC TCC A-3´) that respectively yield 733 bp and 611 bp products were used (Xiao *et al*., 1999; Silva *et al*., 2013). Reactions were performed using Taq DNA Polymerase Master Mix RED® (Ampliqon, Odense, Denmark) in a SimpliAmp® thermal cycler (Applied Biosystems, Waltham, MA, USA). The amplified products at the second nest were detected by electrophoresis on 2% agarose gels stained with a safe DNA stain (SinaClon, Tehran, Iran). Because of the uncommonly high frequency (i.e. 100%) the nested PCRs were repeated one more time.

It should be noted that to prevent cross-contamination in nested PCR, precautions were taken including the preparation of reagents in separate PCR-UV chambers equipped with independent batches of reagents, micropipette sets, and sterile reagent tubes.

For all reactions, a negative control (water) and DNA of *C. parvum* obtained in a previous study were used.

Sequencing and phylogenetic analysis

Five fecal and three tracheal PCR products from the histopathology-confirmed samples were randomly selected and sequenced bidirectionally in an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Sequence reads were curated manually by removing all primer sequences and compared with those available in the GenBank® database, using the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The phylogenetic analysis was performed by examining the sequences obtained in the present study and those from the GenBank database by Neighbor-Joining method (Saitou and Nei, 1987), and evolutionary analyses were conducted on 1000 bootstrap replications (Felsenstein, 1985) in the MEGA7 software (Kumar *et al*., 2016). The Trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2021) and were in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated.

Statistical analysis

The Chi-square's test was used to determine the associations between infections and the gender or age (under and over six months old) of the pigeons. Differences were considered significant at $P<0.05$. The analyses were performed with IBM SPSS Statistics v.22 software.

Results

Clinical signs associated with gastrointestinal and respiratory systems, which were found in all positive birds were weight loss (100%), diarrhea (46%), dyspnea (19%), nasal and ocular mucosal discharge (11% and 6%, respectively), and chronic regurgitation (9%). Enteritis (35%) was the most common finding followed by hyperemia (16%) or hemorrhage (4%), hyperemic trachea containing foamy fluid (14%), air sacculitis with foamy or purulent fluids (8%), purulent bronchopneumonia with focal consolidation, degeneration, and necrosis (5%), and enlarged bursa of Fabricius that showed focal hemorrhages (2%).

At light microscopy, purple *Cryptosporidium* oocysts with a diameter of about 4-5 µm were detected in MZN-stained fecal smears of 62 pigeons (62%, 95% CI: 0.61-0.63) (Fig. 2A).

Cryptosporidium-like organisms measuring 3-8 µm were observed in the epithelial layer of the trachea (78%, 95% CI: 0.70-0.86) (Figs. 2B, C, and D) and on the apical epithelial surfaces of the small intestine (84%, 95% CI: 0.77-0.91) of the pigeons (Figs. 2E and F). In 23 pigeons (23%, 95% CI: 0.15-0.31), simultaneous tracheal and intestinal infection was recorded. The main histopathological lesions in the small intestine were hyperemia, villous atrophy and fusion, dilatation of intestinal crypts, irregular epithelial hyperplasia and

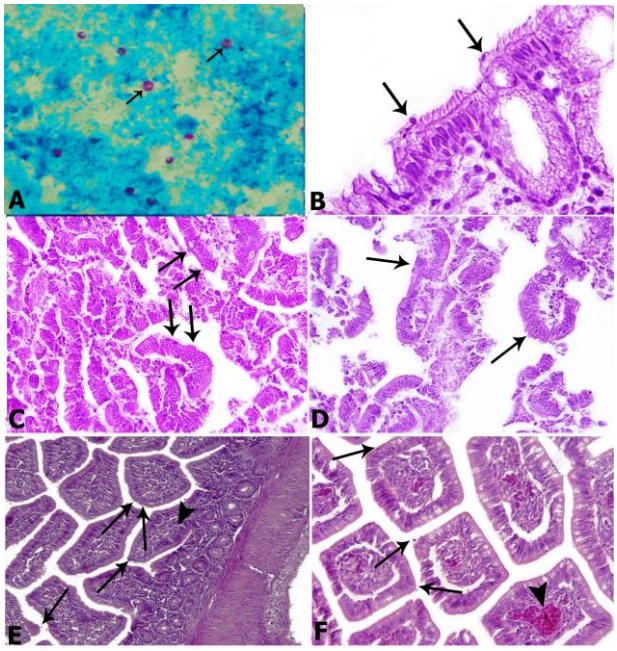

Fig. 2: Rock domestic pigeon naturally infected with *Cryptosporidium* spp. (**A**) Purple *Cryptosporidium* oocysts-like organisms (arrows) measuring 4-5 µm in diameter in fecal smears (modified Ziehl-Neelsen staining, oil immersion), (**B**) *Cryptosporidium* oocysts-like organism (arrows) in the brush border of the mucosal surface of the trachea, (**C**, **D**, **E** and **F**) The small intestine exhibited *Cryptosporidium* oocysts-like organisms (arrows) on the mucosal brush border of the epithelial associated with lymphoplasmacytic infiltration (E: arrowhead) and capillary hyperemia (F: arrowhead). The endogenous developmental stage of the parasite was observed in the affected intestinal villi (F: upper and lower arrows), (H&E staining, scale bars of **A** and **B** = 10 µm, scale bars of **C**, **D**, and **F** = 30 µm, and scale bar of **E** = 60 µm)

sloughing, and diffused mixed inflammatory cell infiltration in the lamina propria with dominant lymphocytes and plasma cells and fewer heterophils.

All the pigeons were positive in PCR results for both fecal and tracheal samples. Consensus sequences for eight sequenced samples displayed 98.19-100% nucleotide identity with those available in the GenBank® database. In the fecal materials, *C. parvum* was confirmed in four and *C. meleagridis* in one pigeon whereas *C. parvum* was confirmed in only three tracheal specimens. The representative sequences of the parasites detected in this study were deposited in the GenBank® database under the accession numbers OP601566, OP601567, OP601568, OP601569, OP601570, OP601571, and OP601572 for *C. parvum* and OP602326 for *C. meleagridis.* The molecular identifications were supported by the distinct separation of species-specific clades inferred from the phylogenetic analyses (Fig. 3).

There were no significant differences in the separate intestinal and tracheal infection rates and also simultaneous infection between males (45, 38, and, 14 out of 50, respectively) and females (39, 40, and 9 out of 50, respectively) (χ^2=2.65, P=0.10; χ^2=0, P=1.00; χ^2=0.50, P=0.47, respectively). Of note, there were significant differences in the separate intestinal and tracheal infection rates in two age categories of over six months old (43 and 36 out of 56, respectively) and under six months old (41 and 42 out of 44, respectively) (χ^2=4.87, P=0.027, and χ^2=9.47, P=0.002, respectively). There was no remarkable difference in the simultaneous infection rate of the intestine and trachea in the above mentioned two age categories (9 and 14 out of 56 and 44, in the adults and juvenile, respectively) (χ^2=3.41, P=0.065).

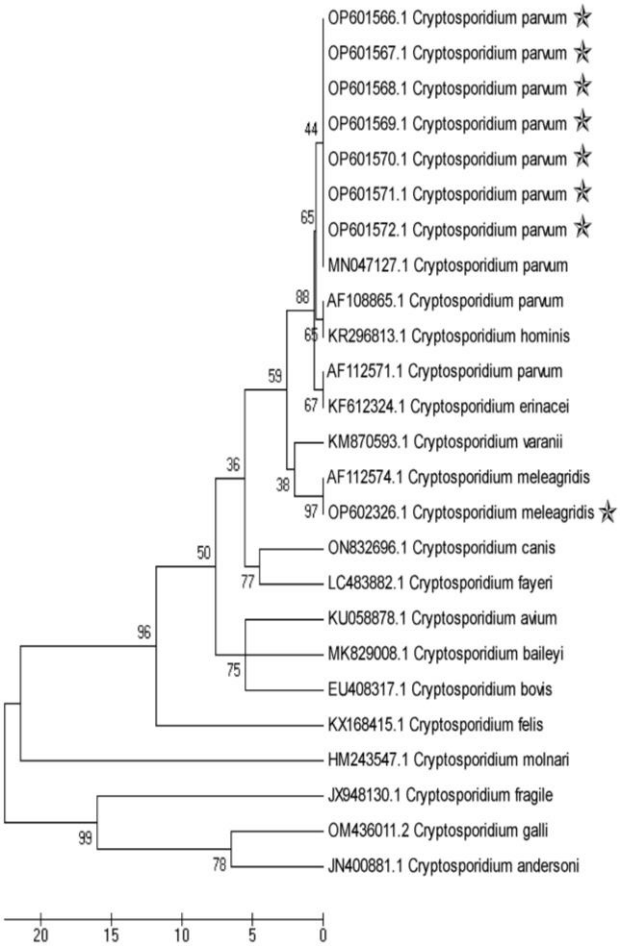

Fig. 3: Phylogenetic relationship of the *18S rRNA* gene of *Cryptosporidium* sequences detected in this study and other *Cryptosporidium* isolates deposited in GenBank database. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 149.46093750 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the base number of difference method per sequence. The analysis involved 25 nucleotide sequences. The Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 464 positions in the final dataset. Evolutionary analyses were conducted in MEGA7. The stars indicated the sequences obtained in this study

Discussion

The high frequency of intestinal and respiratory cryptosporidiosis which was observed in the present

study, could be due to the selection of sick pigeons. The identification of *C. parvum* and *C. meleagridis* indicates that pigeons in Iran are exposed to and might be infected with these zoonotic parasites that poses a risk not only to pigeons and other animal populations but also to humans. In fact, the focus of the present study was on the frequency of *Cryptosporidium* in pigeons with non-specific clinical symptoms. Of the relatively few reports available on *Cryptosporidium* in pigeons, several are case reports (Özkul and Aydin, 1994; Rodriguez *et al*., 1997) that have used fecal smear microscopic examination. Indeed, at the fecal examination of pigeons (n=102) in a northeastern region of Iran the overall prevalence of cryptosporidial infection was 2.9% ranging from 2.3% to 3.4% in adults and in nestlings, respectively (Radfar *et al*., 2012). In another study in Tehran, oocysts were diagnosed in 1/40 (2.5%) of pigeons (Mirzaghavami *et al*., 2016). In studies from different countries based on PCR diagnosis, cryptosporidiosis in pigeons was reported at rates lower than those reported herein, ranging from 0.82% to 4.8% in Guangdong and Henan Provinces of China (Qi *et al*., 2011; Li *et al*., 2015), to 7% in Brazil (Oliveira *et al*., 2017) and 25% in Thailand (Koompapong *et al*., 2014). The reported differences in infection rates may be associated with the age and health status of the birds, geographical region, sample size, hygiene measurements, diagnostic methods, *etc*.

Weight loss and diarrhea were the most common clinical signs which were observed in pigeons which were included in this study. In particular, diarrhea associated with cryptosporidiosis in pigeons was previously described (Özkul and Aydin, 1994; Rodriguez *et al*., 1997). It has also been reported that cryptosporidiosis is associated with yellow watery diarrhea, weight loss, dehydration, and weakness in 40% of farmed pigeons and mortality in 5% (Rodriguez *et al*., 1997). Invasive stages of *Cryptosporidium* in the small intestine, caecum, colon, cloaca, and bursa are also detected in the necropsy of some birds (Rodriguez *et al*., 1997). Invasive stages of *Cryptosporidium* were detected in the small intestine of a pigeon that had been depressed and presenting diarrhea (Özkul and Aydin, 1994). However, it seems that in pigeons similar to other avian species, *Cryptosporidium* infection may exhibit unspecific clinical symptoms related to intestine, respiratory tract and/or renal systems, inducing a wide range of signs such as weight loss, depression, diarrhea, coughing, tracheitis, airsacculitis, and accumulation of mucus in the respiratory airways (Nakamura and Meireles, 2015). In addition, hypertrophy or atrophy of the bursa of Fabricius has been reported, in some *Cryptosporidium* infected birds (Goodwin *et al*., 1996). Although several studies have reported *C. parvum* from asymptomatic birds and the role of birds is postulated mainly as mechanical transporters (Quah *et al*., 2011; Shahbazi *et al*., 2020), *C. parvum* was associated with catarrhal enteritis, in an outbreak of cryptosporidiosis in a collection of stone curlews (*Burhinus oedicnemus*) in Dubai, as numerous developmental stages of the parasites were observed at the mucosal surfaces of infected birds (Zylan *et al*., 2008). Experimental infection studies are needed to better elucidate the organs which get involved during cryptosporidiosis in pigeons.

This is the first study that reports tracheal cryptosporidiosis in pigeons. Similar to our observations, enteritis, hyperemia, intestinal distension, and the presence of evolutionary stages of the parasite in the epithelium of the small intestine have been common findings in avian intestinal cryptosporidiosis (Özkul and Aydin, 1994; Nakamura and Meireles, 2015; Shahbazi *et al*., 2020). However, respiratory system complications and the possible transmission of the parasites via nasal and oral routes during cryptosporidiosis in pigeons have not been addressed, so far. Examination of oral swabs and nasal and ocular discharges in infected birds will shed light on the hypothesis that shared drinking water and feeding sources of offspring and adult pigeons might play a role as infection transmission routes.

Though no significant difference was observed in the infection rates between male and female pigeons, there were remarkable differences between the two age categories, being higher in the younger pigeons. Similarly, the previous reports proposed age-associated differences between *Cryptosporidium* infection in pet birds and broiler chickens, which was more prevalent in young and immunocompromised birds (Qi *et al*., 2011; Shahbazi *et al*., 2020).

Consensus sequences of detected parasites revealed infection of pigeons with *C. parvum* and *C. meleagridis*. It has been suggested that pigeons play a role in the transmission of human-pathogenic cryptosporidia. So far, *C. hominis* (Abreu-Acosta *et al*., 2009), *C. parvum* (Oliveira *et al*., 2017) and *C. meleagridis* (Qi *et al*., 2011, Koompapong *et al*., 2014; Li *et al*., 2015) have been reported in fecal specimens of domestic pigeons in Spain (Abreu-Acosta *et al*., 2009), Brazil (Oliveira *et al*., 2017), Thailand (Koompapong *et al*., 2014), and China (Qi *et al*., 2011; Li *et al*., 2015). Indeed, *C. parvum* is one of the most common *Cryptosporidium* species in humans and may infect several wild and domestic avian species (Xiao and Feng, 2008; McEvoy and Giddings, 2009) like chickens or turkeys, which can be completely asymptomatic (McEvoy and Giddings, 2009; Helmy *et al*., 2017; Shahbazi *et al*., 2020). This renders these species suitable reservoirs for environmental contamination. *Cryptosporidium meleagridis*, another species detected in the current study, commonly infects the small and large intestines of Columbiformes, Galliformes, Passeriformes, and Psittaciformes birds in Africa, Asia, Europe, Oceania, North America, and South America (Nakamura and Meireles, 2015). Respiratory infection of farmed partridges presenting clinical signs such as diarrhea and coughing, morbidity of 60-70% and mortality of 50%-89% was associated to *C. meleagridis*, in a red-legged partridge game farm in Cataluña, northeast Spain (Pagès-Manté *et al*., 2007). Considering that domestic pigeons are one of the most common birds worldwide and both *C. parvum* and *C. meleagridis* have frequently been reported from human

patients globally (Rafiei *et al*., 2014; Feng *et al*., 2018; Ghafari *et al*., 2018) increasing awareness regarding possible transmission of zoonotic *Cryptosporidium* species from pigeons, and in particular the management of excreta of pigeons in public places are advocated.

As a limitation of this study, PCR was conducted solely on fecal and tracheal tissue, and histopathological analysis was limited to intestinal and tracheal tissues. However, avian cryptosporidioses are known to affect multiple organs, including the eyes, lungs, kidneys, gastrointestinal tract, and bursa of Fabricius. (Nakamura and Meireles, 2015; Wang *et al*., 2021). Therefore, examination of these tissues by PCR and histopathological methods would shed light on the actual role of pigeons in epidemiology of cryptosporidiosis, in future studies.

This study demonstrated for the first time the presence of *C. meleagridis* and *C. parvum*, two common zoonotic *Cryptosporidium* species in domestic pigeons in Iran. The finding of *C. parvum* associated with cryptosporidiosis in birds is important because it has been speculated that birds are just a mechanical vector for *C. parvum*. The high frequency of intestinal and respiratory cryptosporidiosis reported herein could be due to the selection of sick pigeons. Of note, *C. meleagridis* and *C. parvum* are the common *Cryptosporidium* species in humans that were previously recognized in wild and domestic birds, and they are proposed as a reservoir for human infections. Therefore, the results indicate that pigeons in Iran are exposed to and could be infected by these zoonotic parasites and may represent a risk for other animal populations and humans.

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Conflict of interest

The authors declare no conflict of interest.

References

Abreu-Acosta, N; Foronda-Rodríguez, P; López, M and Valladares, B (2009). Occurrence of *Cryptosporidium hominis* in pigeons (*Columba livia*). Acta Parasitol., 54: 1-5.

Adang, K; Oniye, S; Ezealor, A; Abdu, P and Ajanusi, O (2008). Ectoparasites of domestic pigeon (*Columba livia domestica*, Linnaeus) in Zaria, Nigeria. J. Parasitol. (Faisalabad), 3: 79-84.

Ditrich, O; Palkovic, L; Stěrba, J; Prokopic, J; Loudová, J and Giboda, M (1991). The first finding of *Cryptosporidium baileyi* in man. Parasitol. Res., 77: 44-47.

Felsenstein, J (1985). Phylogenies and the comparative method. Am. Nat., 125: 1-15.

Feng, Y; Ryan, UM and Xiao, L (2018). Genetic diversity and population structure of *Cryptosporidium*. Trends Parasitol., 34: 997-1011.

Ferrari, ED; Nakamura, AA; Nardi, ARM; Santana, BN; da Silva Camargo, V; Nagata, WB; Bresciani, KDS and Meireles, MV (2018). *Cryptosporidium* spp. in caged exotic psittacines from Brazil: Evaluation of diagnostic methods and molecular characterization. Exp. Parasitol., 184: 109-114.

Ghafari, R; Rafiei, A; Tavalla, M; Choghakabodi, PM; Nashibi, R and Rafiei, R (2018). Prevalence of *Cryptosporidium* species isolated from HIV/AIDS patients in southwest of Iran. Comp. Immunol. Microbiol. Infect. Dis., 56: 39-44.

Ghiai, MM; Tabe Arjmand, J; Mohammadi, O; Ahmadi, MH and Assad, MEH (2021). Investigation and modeling of energy consumption of tall office buildings in Iran's `hot-arid' and `cold' climate conditions. Int. J. Low-Carbon Technol., 16: 21-34.

Goodwin, MA; Brown, J; Resurreccion, RS and Smith, JA (1996). Respiratory coccidiosis (*Cryptosporidium baileyi*) among northern Georgia broilers in one company. Avian Dis., 40: 572-575.

Harlin, RW (1994). Pigeons. Vet. Clin. North. Am. Small Anim. Pract., 24: 157-173.

Helmy, YA; Krücken, J; Abdelwhab, ESM; von Samson-Himmelstjerna, G and Hafez, HM (2017). Molecular diagnosis and characterization of *Cryptosporidium* spp. in turkeys and chickens in Germany reveals evidence for previously undetected parasite species. PLoS One. 12: e0177150.

Koompapong, K; Mori, H; Thammasonthijarern, N; Prasertbun, R; Pintong, A; Popruk, S; Rojekittikhun, W; Chaisiri, K; Sukthana, Y and Mahittikorn, A (2014). Molecular identification of *Cryptosporidium* spp. in seagulls, pigeons, dogs, and cats in Thailand. Parasite. 21: 52.

Kopacz, Ż; Kváč, M; Piesiak, P; Szydłowicz, M; Hendrich, AB; Sak, B; McEvoy, J and Kicia, M (2020). *Cryptosporidium baileyi* pulmonary infection in immunocompetent woman with benign neoplasm. Emerg. Infect. Dis., 26: 1958-1961.

Kumar, S; Stecher, G and Tamura, K (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol., 33: 1870-1874.

Lallo, MA; Calábria, P and Milanelo, L (2012). *Encephalitozoon* and *Enterocytozoon* (microsporidia) spores in stool from pigeons and exotic birds: Microsporidia spores in birds. Vet. Parasitol., 190: 418-422.

Li, J; Lin, X; Zhang, L; Qi, N; Liao, S; Lv, M; Wu, C and Sun, M (2015). Molecular characterization of *Cryptosporidium* spp. in domestic pigeons (*Columba livia domestica*) in Guangdong Province, Southern China. Parasitol. Res., 114: 2237-2241.

McEvoy, J and Giddings, C (2009). *Cryptosporidium* in commercially produced turkeys on-farm and postslaughter. Lett. Appl. Microbiol., 48: 302-306.

Mirzaghavami, M; Sadraei, J and Forouzandeh, M (2016). Detection of *Cryptosporidium* spp. in free ranging animals of Tehran, Iran. J. Parasit. Dis., 40: 1528-1531.

Nakamura, AA and Meireles, MV (2015). *Cryptosporidium* infections in birds-a review. Rev. Bras. Parasitol. Vet., 24: 253-267.

Nakamura, AA; Simões, DC; Antunes, RG; da Silva, DC

and Meireles, MV (2009). Molecular characterization of *Cryptosporidium* spp. from fecal samples of birds kept in captivity in Brazil. Vet. Parasitol., 166: 47-51.

Ng, J; Pavlasek, I and Ryan, U (2006). Identification of novel *Cryptosporidium* genotypes from avian hosts. Appl. Environ. Microbiol., 72: 7548-7553.

Oliveira, BCM; Ferrari, ED; da Cruz Panegossi, MF; Nakamura, AA; Corbucci, FS; Nagata, WB; Dos Santos, BM; Gomes, JF; Meireles, MV and Widmer, G (2017). First description of *Cryptosporidium parvum* in carrier pigeons (*Columba livia*). Vet. Parasitol., 243: 148-150.

Özkul, I and Aydin, Y (1994). Small-intestinal cryptosporidiosis in a young pigeon. Avian Pathol., 23: 369-372.

Pagès-Manté, A; Pages-Bosch, M; Majó-Masferrer, N; Gómez-Couso, H and Ares-Mazás, E (2007). An outbreak of disease associated with cryptosporidia on a red-legged partridge (*Alectoris rufa*) game farm. Avian Pathol., 36; 275-278.

Prediger, J; Ježková, J; Holubová, N; Sak, B; Konečný, R; Rost, M; McEvoy, J; Rajský, D and Kváč, M (2021). *Cryptosporidium sciurinum* n. sp. (Apicomplexa: Cryptosporidiidae) in Eurasian red squirrels (*Sciurus vulgaris*). Microorganisms. 9: 2050.

Qi, M; Wang, R; Ning, C; Li, X; Zhang, L; Jian, F; Sun, Y and Xiao, L (2011). *Cryptosporidium* spp. in pet birds: genetic diversity and potential public health significance. Exp. Parasitol., 128: 336-340.

Quah, JX; Ambu, S; Lim, YAL; Mahdy, MAK and Mak, JW (2011). Molecular identification of *Cryptosporidium parvum* from avian hosts. Parasitology. 138: 573-577.

Radfar, MH; Asl, EN; Seghinsara, HR; Dehaghi, MM and Fathi, S (2012). Biodiversity and prevalence of parasites of domestic pigeons (*Columba livia domestica*) in a selected semiarid zone of South Khorasan, Iran. Trop. Anim. Health Prod., 44: 225-229.

Rafiei, A; Rashno, Z; Samarbafzadeh, A and Khademvatan, S (2014). Molecular characterization of *Cryptosporidium* spp. isolated from immunocompromised patients and children. Jundishapur J. Microbiol., 7: e9183.

Rodriguez, F; Oros, J; Rodriguez, JL; Gonzalez, J; Castro, P and Fernandez, A (1997). Intestinal cryptosporidiosis in pigeons (*Columba livia*). Avian Dis., 41: 748-750.

Ryan, U (2010). *Cryptosporidium* in birds, fish and amphibians. Exp. Parasitol., 124: 113-120.

Ryan, UM; Feng, Y; Fayer, R and Xiao, L (2021). Taxonomy and molecular epidemiology of *Cryptosporidium* and *Giardia* - a 50 year perspective (1971-2021). Int. J. Parasitol., 51: 1099-1119.

Ryan, U; Zahedi, A and Paparini, A (2016). *Cryptosporidium* in humans and animals-a one health approach to prophylaxis. Parasite Immunol., 38: 535-547.

Saitou, N and Nei, M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4: 406-425.

Santín, M; Trout, JM; Xiao, L; Zhou, L; Greiner, E and Fayer, R (2004). Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. Vet. Parasitol., 122: 103-117.

Shahbazi, P; Aligolzadeh, A; Khordadmehr, M; Farhang, HH and Katiraee, F (2020). Molecular study and genotyping of *Cryptosporidium baileyi* and *Cryptosporidium parvum* from free-range and commercial broiler chickens in Guilan province, Iran. Comp. Immunol. Microbiol. Infect. Dis., 69: 101411.

Silva, SO; Richtzenhain, LJ; Barros, IN; Gomes, AM; Silva, AV; Kozerski, ND; de Araújo Ceranto, JB; Keid, LB and Soares, RM (2013). 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., 135: 551-557.

Sréter, T and Varga, I (2000). Cryptosporidiosis in birds - a review. Vet. Parasitol., 87: 261-279.

Tamura, K; Stecher, G and Kumar, S (2021). MEGA 11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol., 38: 3022-3027.

Taylor, MA; Coop, RL and Wall, RL (2016). *Veterinary parasitology*. 4th Edn., Oxford: Ames, Iowa, Wiley Blackwell. PP: 720-723.

Wang, Y; Zhang, K; Chen, Y; Li, X and Zhang, L (2021). Cryptosporidium and cryptosporidiosis in wild birds: A One Health perspective. Parasitol Res., 120: 3035-3044.

WOAH (World Organisation for Animal Health) (2022). *Cryptosporidiosis, manual of diagnostic tests and vaccines for terrestrial animals* 2022. WOAH: Paris, France, Chapter 3, 10.2.2022. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.10.02_CRYPTO.pdf Accessed 04 July 2023.

Xiao, L; Escalante, L; Yang, C; Sulaiman, I; Escalante, AA; Montali, RJ; Fayer, R and Lal, AA (1999). Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. Appl. Environ. Microbiol., 65: 1578-1583.

Xiao, L and Feng, Y (2008). Zoonotic cryptosporidiosis. FEMS Immunol. Med. Microbiol., 52: 309-323.

Zahedi, A; Paparini, A; Jian, F; Robertson, I and Ryan, U (2016). Public health significance of zoonotic *Cryptosporidium* species in wildlife: critical insights into better drinking water management. Int. J. Parasitol. Parasites Wildl., 5: 88-109.

Zylan, K; Bailey, T; Smith, HV; Silvanose, C; Kinne, J; Schuster, RK and Hyland, K (2008). An outbreak of cryptosporidiosis in a collection of stone curlews (*Burhinus oedicnemus*) in Dubai. Avian Pathol., 37: 521-526.