Detection of *Cryptosporidium* spp. in free ranging animals of Tehran, Iran

Mehran Mirzaghavami¹ · Javid Sadraei¹ · Mehdi Forouzandeh²

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Abstract *Cryptosporidium* is a world widely distributed parasite which comparatively has a high prevalence in developing countries. The zoonotic potential of some *Cryptosporidium* species has made the cryptosporidiosis a significant concern to physicians and veterinarians. The occurrence and zoonotic potential of *Cryptosporidium* species in probable reservoir hosts for man infections was determined by examining faeces of symptomatic and asymptomatic animals. The aim of this study is to screen the presence of *Cryptosporidium* in fecal sample of free ranging animals in Tehran using Ziehl–Neelsen staining method. The findings indicate that *Cryptosporidium* are present in 9/50 (18 %) stray cat (*Felis catus*), 12/50 (24 %) hooded crows (*Corvus cornix*), 23/180 (12.7 %) rat (*Rattus norvegicus* and *R. rattus*) and 1/40 (2.5 %) pigeons (*Columba livia*). This investigation confirms the potential role of rats, cats, crows and pigeons for zoonotic transmission of human cryptosporidiosis and they must be considered as reservoir hosts which can endanger public health.

Keywords *Cryptosporidium* · Free ranging animals · Cat · Crow · Rat · Pigeons · Iran

Introduction

Cryptosporidiosis is caused by a protozoa belonging to genus *Cryptosporidium* and Apicomplexa phylum which can affect various hosts including mammals, birds, fish and reptiles (Ajonina et al. 2012). Depending on the host immunity system the clinical signs of cryptosporidiosis can manifest a variety of range from an acute self-limiting diarrhea to a chronic life-threatening infection in immunocompromised individuals such as AIDS patient. Clinical signs of gastrointestinal cryptosporidiosis in avian and mammalian hosts include diarrhea, lethargy, apathy and depression, anorexia, lower pigmentation, growth retardation, and mortality (Alvarez-Pellitero and Sitjà-Bobadilla 2002). Infection transmission usually occurs through the fecal-oral route or by indirect contamination of food, water supplies and tools with infective distributed oocysts in the environment (Robertson and Gjerde 2000). Accordingly more than 20 species and also over 50 genotypes of *Cryptosporidium* have been recorded. Some of these species and genotypes are host specific, but some of them such as *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, and *C. muris* have been detected in human hosts (Wang et al. 2011). Animals can be accounted as a probable zoonotic reservoir for human infection or vice versa (Anderson et al. 1982; O'Donoghue 1995). The aim of current study is to examine the presence of *Cryptosporidium* oocysts in the feces of the free-ranging animals coexisted in human habitat. Further studies can be conducted based on the results of the present research to identify the role of these hosts in food and water supply contamination and their potentiality of zoonotic infection, particularly among population at risk including immunodeficient individuals.

✉ Javid Sadraei
sadraei.j@modares.ac.ir

¹ Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
² Department of Medical Biotechnology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Table 1 Detection of *Cryptosporidium* oocysts in free ranging animals in Tehran

Sample	No.	Positive (%)	Oocyst dimensions	Part name in Fig. 1
<i>Corvus cornix</i>	50	12 (24)	$5.0 \pm 0.3 \times 4.6 \pm 0.3$	A
<i>Columba livia</i>	40	1 (2.5)	5.39×4.0	B
<i>Rattus norvegicus</i> and <i>R. rattus</i>	180	23 (12.7)	$9.5 \pm 0.7 \times 5.5 \pm 0.3$	C
<i>Felis catus</i>	50	9 (18)	$6.9 \pm 0.2 \times 4.6 \pm 0.3$	D

Fig. 1 Oocyst of *Cryptosporidium* spp., stained by the modified Ziehl–Neelsen staining technique. **a** Hooded crow (*C. cornix*), **b** pigeon (*C. livia*), **c** rat (*R. norvegicus* and *R. rattus*) and **d** cat (*F. catus*)

Materials and methods

Study period and sample collection

This study was performed based on cross sectional design from June 2012 to July 2013 in different regions of Tehran, Iran. The samples collected from different areas after permission from the environmental organization.

Fecal samples were collected after trapping from 50 stray cats (*Felis catus*), 50 of hooded crows (*Corvus cornix*), 180 rats (*Rattus norvegicus* and *R. rattus*) and 40 pigeons (*Columba livia*). Samples were transferred to the plastic falcon tubes containing 2/5 % dichromate potassium and kept at 4 °C, separately.

Sample procedure

Each fecal sample was passed through gauze mesh and a centrifugal sedimentation concentration method with water-ether solution was performed then the tube was shaken and a direct smear was prepared from the resulting fecal suspension. Afterward the smears were air dried and fixed by methanol. Subsequently the smears were stained via modified Ziehl–Neelsen technique and were

investigated microscopically under 1000× magnification for the detection of *Cryptosporidium* oocysts. If one oocyst was seen with the correct morphological characteristics including shape, size, internal structure, and optical properties the sample was considered positive for cryptosporidiosis.

Morphological analysis

The size and morphology of detected oocysts were estimated by light microscope equipped with a calibrated ocular micrometer (de Oliveira Lemos et al. 2012).

Results

Microscopic observation of all the prepared smears from the fecal samples shown that *Cryptosporidium* oocysts were found in 9/50 (18 %) stray cat (*F. catus*), 12/50 (24 %) hooded crow (*C. cornix*), 23/180 (12.7 %) of rats (*R. norvegicus* and *R. rattus*) and 1/40 (2.5 %) pigeons (*C. livia*). The dimension of *Cryptosporidium* spp. isolates oocysts are summarized in Table 1.

Discussion

In the current study we examined the presence of *Cryptosporidium* oocysts in the fecal samples of 50 stray cats, 50 crows, 180 rats, and 40 pigeons. Although the similar works have been done by the other researchers during the recent years the detection of *Cryptosporidium* in Corvidae family has no precedence in Iran and from this view of points this research has a novel dimension. In a study conducted by Bahrami, the prevalence of *Cryptosporidium* was reported 13 % in wild rats in Tehran (Bahrami et al. 2012a, b). In a study conducted by Pakdel on the mice and rats in Kermanshah there is no report about the gastrointestinal protozoa (Pakdel et al. 2013). In a study on the endoparasites of domestic cats with diarrhea in Rio de Janeiro by Lemos 8.33 % occurrence of *Cryptosporidium* was reported (de Oliveira Lemos et al. 2012). In similar researches conducted on the endoparasites of stray cats in Kashan *Cryptosporidium* was not pointed out (Arbabi and Hooshyar 2009). Another microscopic study by Borkataki indicated 4 % prevalence of *Cryptosporidium* among the stray cats in Jammu region in India (Borkataki et al. 2013). Investigation about cryptosporidiosis in birds in Iran have been mostly done on the turkey, poultry, ostrich and borealis (Banani et al. 2000; Shemshadi et al. 2014). But recently some Iranian researchers have shifted on the free-living birds ecto-endoparasites (Halajian et al. 2011; Pirestani et al. 2013). Pathological study of parasitism in racing pigeons by Bahrami (2012a, b) shown 1.78 % prevalence of *Cryptosporidium* (Mohammad et al. 2013). Prevalence of parasites in urban birds in Khorramabad city indicated 2.7 % cryptosporidiosis among pigeons (Badparva et al. 2014). In a survey on parasites of domestic pigeons in South Khorasan province by Radfar, *Cryptosporidium* oocysts were identified in 2.94 % of the samples (Radfar et al. 2011). Results of parasites investigation in large-billed crow in Malaysia demonstrated 33.96 % presence of *Cryptosporidium* oocysts in the fecal samples by using acid fast staining method (Yong et al. 2008). *Cryptosporidium* infection in cats are usually diagnosed with no clinical symptoms as well as with normal stool which contains few oocysts (Abe et al. 2002; Peregrine 2007). Depending on the method of detection prevalence of *Cryptosporidium* in cats varies from 0 to 29 % (Lucio-Forster et al. 2010). The observed result in this study is to some extent consistent with other results reported by previously conducted researches in Iran. Cryptosporidiosis in birds namely consists of three major forms, which are intestinal, respiratory and renal form and it can be fetal in severe cases (Rohela et al. 2005). It is worthy to mention that oocysts observation is more or less a difficult task

and skilled technicians is needed to make an accurate diagnosis because it can be mistaken for yeasts, debris and the like. On the contrary, if the oocysts are failed to be stained correctly false negative results are inevitably possible. Although morphological aspects of the oocysts could be an useful way to suggest the probable species of the some *Cryptosporidium* genus, it is not a completely reliable tool but molecular techniques are able to determine the exact species and subtypes of the *Cryptosporidium* oocysts (Fall et al. 2003). On the other hand in some cases because of the low number of oocysts in samples and the small amount of extracted DNA, in the nested-PCR products, a pale band was demonstrated after gel electrophoresis and therefore some *Cryptosporidium* species was not identifiable via sequencing reaction in positively detected samples (Nakamura et al. 2009).

Conclusion

The findings of the current study revealed the public health significance of the free-ranging animals coexisted in humane habitat in terms of zoonotic transmission of the cryptosporidiosis infection. Since there is no effective cure for this infection therefore prevention of water and food supplies contamination with discarded oocysts seems the most essential issue. Additionally, the exposure of susceptible individuals with these animals in public zones must be reduced to decrease the possibility of infection transmission. On the other hand these animals might be considered as a threatening factor for the livestock and poultry industries. Further investigations to determine the molecular characterization of *Cryptosporidium* species and genotypes and the status of this parasite in different regions seem necessary.

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Compliance with ethical standards

Conflict of interest None.

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