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A survey on *Toxocara cati* eggs on the hair of stray cats: A potential risk factor for human toxocariasis in Northeastern Iran

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ABSTRACT

The objective of this study was to investigate the presence of *Toxocara* eggs on the hair of stray cats. The total number of stray cats trapped and included in the trial was 167 that were collected weekly from different residential areas of Mashhad, in northeastern Iran, from November 2016 to December 2017. Among the 167 cats, 18 (10.8%) of them were positive to *T. cati* eggs in their hair. In the positive cats, 7 (39%) were adult, 1 (6%) was juvenile and 10 (55%) were kittens. Overall, the mean number of eggs from positive cats was 3.9 ± 1.7 eggs per gram (epg) of hair per cat with an average of 3.1 ± 1.4 in adults, 4.9 in juveniles and 4.3 ± 1.6 in kittens. In total, 39.9% of the eggs recovered were non-viable 35.5% were viable, 22.2% were embryonating and 2.3% were embryonated which embryonated eggs were found only in juveniles. Based on our data, kittens were responsible for 61.7% of the total number of eggs. The age of the cat was found to be an important risk factor associated with parasitic infection.. This study showed that cat hair contaminated by *T. cati* eggs in different developmental stages represents of potential source for human toxocariasis.

1. Introduction

Toxocara cati, the most prevalent intestinal roundworm of cats and other feline hosts, is considered to be one of the most commonly reported zoonotic helminth infection worldwide responsible for human toxocariasis. Infective ova excreted by this highly fecund parasite are not immediately infective and contaminate the environment extensively exposing paratenic hosts, and humans, to the risk of infection [1]. Human infection may be acquired by ingesting viable, embryonated eggs of this Ascarid nematode from contaminated sources such as the coat of cats or soil, and can cause the well-characterized syndromes including visceral larva migrans (VLM), ocular larva migrans (OLM) and covert toxocariasis (CT), and only a few larvae are needed to cause disease [2,3]. It has been revealed that stray cats may represent a major source of *Toxocara* spp. eggs due to the high numbers of *Toxocara* they harbor. This is presumably due to lack of anthelmintic treatment compared to owned cats. In Iran, cats are often reared at homes as a pet or exploited as a predator of rats but for some reasons they may be lost or abandoned and live as stray cats. These stray cats live freely in urban and rural areas, and tend to discharge *Toxocara* spp. eggs into the environment (Table 1).

An overarching need for all aspects of toxocariasis is the collection of appropriate epidemiological data [3]. Several coproscopic investigations have been performed in different regions of the world for *Toxocara* spp. infection in stray cats which is presented in Table 1. In the majority of these cases little is known about the potential routes of transmission for *Toxocara* spp. to humans. However, there is a lack of good-quality data on the relative risk of human infection from stray cats and the degree of environmental contamination in any given country. To improve surveillance of toxocariasis in areas at risk in different parts of the world, data are required about environmental contamination and epidemiological parameters from definitive hosts. A few studies have been performed in order to evaluate the hair contamination of the *Toxocara* eggs on the cats. Recently, infective eggs have been found in the hair of cats suggesting that direct contact with the coat of a contaminated cat could be an additional route of transmission [4,5].

To further investigate the potential risk of human infection via direct contact, the present study was designed to investigate the hair contamination of stray cats with *Toxocara* eggs in different sex and age groups in Northeast Iran.

2. Materials and methods

2.1. Study area

This study was performed based on cross sectional design in

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Table 1
Prevalence of *Toxocara cati* in stray cats in different regions of Iran and other country by necropsy and fecal analysis.

Region	Place	Prevalence (%)	Reference
Iran	Northeastern Iran	28[a]	Borji et al.(2011)
	Northwestern Iran	78[a]	Hajipour et al. (2016)
	South of Iran	26.7[a]	Mikaeili et al. (2013)
	Central of Iran	13.3[a]	Mohsen & Hossein, (2009)
	Northern Iran	44[a]	Sharif et al., 2007
	Northwestern Iran	86.3[a]	Yakhchali et al. (2017
	South of Iran	42.6[a]	Zibaei et al.(2007)
Other countries	Northern Germany	27.1[b]	Becker et al.(2012)
	Central Spain	11.7[b]	Montoya et al. (2018)
	Netherlands	27[b]	Nijsse et al. (2015)
	Argentina	61.2[b]	Sommerfelt et al. (2006)
	Northern Italy	33.1[b]	Spada et al.(2013)
	Greece	24.2[b]	Symeonidou et al. (2018)
	Poland	27.9[b]	Szwabe &Blaszkowska (2017)
	Portugal	38.3[b]	Waap et al.(2014)

[a] Necropsy.
[b] Fecal analysis.

Mashhad city in the northeast of Iran. This city with a population of almost 2.5 million inhabitants is the second largest city in the country which attracts more than 20 million tourists and pilgrims every year. Mashhad is located at 59.35° east longitude and 36.20° latitude, in the valley of the Kashaf River near Turkmenistan border, between the two mountain ranges of Hezar-masjed and Binalood with an average annual precipitation of 250 mm.

2.2. Sampling

One hundred sixty seven stray cats (65 males and 102 females) were trapped alive and collected weekly from different residential areas of Mashhad between November 2016 and December 2017 with permission from appropriate authorities from the Iranian Environmental Health Organization. Trapping using baited cage-traps with tinned fish was undertaken in this area. Then, stray cats were transferred to the Veterinary Hospital, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, and kept individually in cages and an identification number was allocated to each animal. The date of sampling, location, sex and age of each cat were recorded. Based on their dental development and maturation of genital structure and body size, cats were divided as follows: 114 (68%) adults (age > 12 months), 13 (8%) juveniles (age7-12 months) and 40 (24%) kittens (age ≤ 6 months).

2.3. Collection of hair samples and egg detection

Cats were anaesthetized by intra-muscular injection of ketamine (20–25 mg/kg) and xylazine (0.5 mg/kg) and hair samples were clipped from the perianal region and the underside of the tail and mixed together. The individual samples were kept in a zipplock polyethylene bag, Labeled with the cat's ID number. The hair samples were then stored at 4°c until processing which was carried out within 1 week maximum. Hair were sampled so as not to be stained with feces were weighed within the weight range of 0.1 to 0.7 g with a mean value of 0.4 g.

Eggs were recovered from the hair using the technique previously described by Wolfe and Wright [6], with some modifications. Hair samples were washed twice in 40 mL of water with two drops of Tween 20, followed by shaking for 10 min and poured through a 350 µm sieve. The obtained liquid was poured into a sedimentation cup at a temperature of 5 ∘C for 12 h. The sediment was collected using Pasteur pipettes and centrifuged at 800 × g for 15 min. After displacing the supernatant, 70 µL was taken from the sediment and the sample was examined using a compound light microscope under ×10 and ×40 magnification.

The eggs were classified into four groups depending on the stage of development: nonviable (egg wall disrupted or egg not intact), viable (intact egg with contents), embryonating egg (egg with two or more cell divisions) and embryonated (containing a larva). Eggs containing visible larvae which had not fully matured were classified as embryonating [7].

2.4. Fecal samples collection and Coprological examination

Besides collecting hairs, feces from all cats were also collected rectally into clean, sterile 5 ml specimen bottles. Samples were examined immediately using formalin-ether sedimentation method followed by microscopy at ×40 magnification. *T. cati* eggs were identified according to the keys Yamaguti [8] and Soulsby [9]. Samples were designated as positive if one or more *T. cati* ova were detected or negative if no ova were detected.

3. Necropsy

Positive cats to *T. cati* ova were humanely euthanized using sodium thiopental (12.5 mg/kg) and potassium chloride (1–2 mmol/kg), intravenously and autopsied within 1 h of euthanization. During the necropsy, the abdominal cavity was opened and small intestine was removed and the intestinal contents were examined for the presence of *Toxocara* adult worms. After identification of *Toxocara* by appropriate keys Yamaguti [8] and Soulsby [9], the number of *T. cati* in each cat was recorded.

4. Statistical analysis

Pearson's chi-squared test (χ^2) was used to analysis the effect of age, sex and sample site on the infection status of the animal. In addition, one-way ANOVA was used to compare differences between the number of eggs found on adults, juveniles and kittens. Pearson's correlation was used to assess the relationship between egg and helminth burdens. Data were analyzed and statistical comparisons were performed using SPSS 16.0.

5. Results

5.1. Hair analysis

Among 167 cats included in the investigation, 18 (10.8%) of them had *T. cati* eggs in their hair. In the positive cat group, 7 (39%) were adults, 1 (6%) was juvenile and 10 (55%) were kittens. The sex ratio of infected cats was 10 (55%) females and 8 (45%) males.

Overall, the average number of eggs found on positive hair samples was 3.9 ± 1.7 with a median of 2. The mean EPG number found on egg-positive adults was 3.1 ± 1.4 with a median of 2, for egg-positive juveniles, 4.9 with a median of 1.6 and for egg-positive kittens 4.3 ± 1.6 with a median of 3 (Table 2). Totally, 39.9% of the eggs recovered were non-viable, 35. 5% were viable, 22.2% were embryonating and 2.3% were embryonated. The latter was found only in the juveniles.

5.2. Fecal analysis

Fecal samples were collected from 167 cats, 20 (11.9%) were positive for *T. cati* eggs. Eighteen of 20 (90%) cats had eggs in their hairs and the remaining cats (147) had no eggs in their feces and hairs as well. Moreover, necropsy findings of infected cats and numbers of *T. cati* found in 20 infected cats are presented in Table 3.

Table 2
Densities of *Toxocara cati* eggs per gram of hair and classification observed on the hair of stray cats from Northeast of Iran. This table concerns the current study.

	Non-viable	Viable	Embryonating	Embryonated	All egg
All cats, n = 18					
Mean ± S.E.M	1.58 ± 0.87	1.4 ± 0.65	0.88 ± 0.1	0.09 ± 0.02	3.95 ± 1.74
Median	3	2	1	1	2
Total number of eggs found	28	25	16	2	71
Kitten, n = 10					
Mean ± S.E.M	0.78 ± 0.3	2.19 ± 1.54	1.42 ± 0.67	0	4.39 ± 1.6
Median	1	2	46	0	3
Total number of eggs found	8	21	14	0	43
Juvenile, n = 1					
Mean ± S.E.M	0	1.64	1.64	1.64	4.92
Median	0	1.64	1.64	1.64	1.64
Total number of eggs found	0	2	2	2	6
Adults, n = 7					
Mean ± S.E.M	2.94 ± 1.3	0.25 ± 0.04	0	0	3.19 ± 1.4
Median	3	1	0	0	2
Total number of eggs found	20	2	0	0	22

Mean and median values are all per gram of hair.

Table 3
Analysis of factors influencing mean number of eggs on hair. This table concerns the current study.

Factor	d.f	F –ratio	probability
Site of hair	1	0.34	0.567
Sex	1	0.067	0.876
Age	3	44.65	< 0.0001

d.f: degrees of freedom.

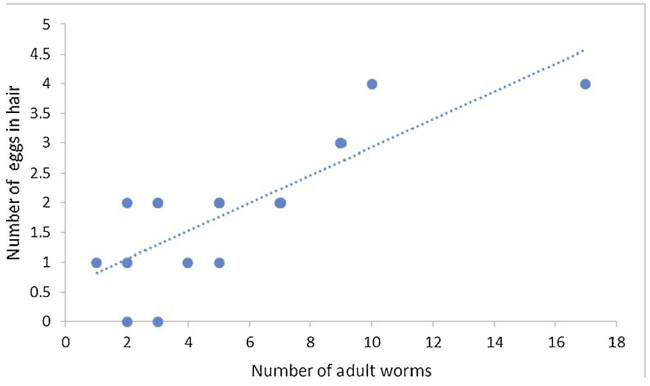

Fig. 1. Spearman correlation analysis of number of adult worms versus numbers of eggs in hair in stray cats (r = _0.83, p < 0.001).

Based on Pearson's chi-squared test, there was no significant difference in infection rate between male and female animals (F = 0.067.; P = 0.876) and the hair site (F = 0.34; P = 0.567). However, the age of the cats was found to be an important risk factor associated with *Toxocara* infection and kittens being more likely to be infected with *T. cati* (F = 44.65; P = 0.001). In addition one way ANOVA analysis, revealed that number of *Toxocara* eggs in kittens was significantly higher than those in adults and juveniles (p < 0.05; Table 2). Moreover, there was a strong positive correlation between the number of worms found in the intestines and the number of eggs found on the hair (r = 0.83, p < 0.001, Fig. 1).

6. Discussion

The presence of *T. cati* on cat's hair highlighted a risk for public health due to the zoonotic character of the parasite. The results obtained in this study indicated that in addition to the reports of *Toxocara* spp. eggs being recovered from feces and in public places, their presence in the hairs of feline is a potential risk factor for the transmission of this parasite to other animals and humans.

So far, only a few studies were performed on the prevalence of *Toxocara* eggs in cat hair [5,4,10]. In our results, prevalence was lower than what was found in Turkey and Mexico [5], but, it was higher than the study in a population of owned cats in the Netherlands [4]. Higher prevalence in stray cats as compared to owned cats is most likely attributable to the lack of anthelminthic treatment given to these animals. Comparatively, results of dog hairs in different regions of the world are also presented in Table 4. As seen from Table 4, most of the research looking for eggs in hairs have been carried out in the dog's hair and a few studies have been performed on the cat's hair.

The results of the present study suggest that kittens are more likely to harbor eggs on their hair than adult cats (more than one year of age). The age of the cat was found to be an important risk factor associated with parasitic infection. These findings were similar to those obtained in other studies described in Table 4. It is probable that infection can occur at any age, either by ingestion of embryonated eggs or tissues containing the larvae, although the highest incidence of infection occurs in kittens. The high *T. cati* prevalence in kittens are associated with the life cycle of the parasite which involves transcolostral transmission, while resistance develops to the parasites in older cats [3].

In the present study, there was no significant difference in the presence of *Toxocara* eggs on cat's hair between females and males. This was in agreement with the findings of previous studies [11–13] who found no difference in the intensity of infection in male and female cats. Sex seemed to have no effect on the prevalence of parasitism, and the only effect of neutering was on the occurrence of Ascarid infection.

The majority of cats in all three-age classes in the present study were positive at concentrations of nearly 71.2 eggs per gram of hair. The number of *T. cati* eggs per gram of hair in this study seemed to be much higher than those collected from the soil samples [14]. In some reports, it has been suggested that the EPG of *Toxocara* in hair of infected animals is much higher than in soil [14–18]. Therefore, ingesting *T. cati* eggs via direct contact from cats may be easier than from soil as indicated by the data in this study. Moreover, the existence of embryonated eggs in some of these hair samples encompasses direct contact with these cats and may be more dangerous than soil contamination for the transmission of toxocariasis in humans.

Data analysis of necropsy finding by Pearson's correlation showed a strong correlation between the number of helminths and the number of eggs on the hair of stray cats (r = 0.83, p < 0.001). Additionally, in two stray cats which were infected based on fecal analysis, we did not find *Toxocara* eggs in their hairs. A possible explanation for this finding may be that cats lick their fur more intensively and thus may find it easier to remove any present eggs.

Table 4
A comparison of numbers of *Toxocara* spp. eggs recovered from hair samples from stray cats and those recovered from dog hairs.

Host	Place	Prevalence (%)	Total number eggs/gr hair	Reference
Stray cats	Turkey	22	28	Oge et al.(2006)
	Netherlands	3.4	28	Overgaauw et al.(2009)
	Mexico	41.7	nd	Rojas et al.(2017)
Stray dogs	Brazil	24	614.8	Amaral et al.(2010)
	Vietnam	56.3	nd	Anh et al.(2016)
	Turkey	21.56	62	Aydenizöz-Ozkayhan et al.(2008)
	Egypt	26.6	77.6	El-Tras et al.(2011)
	Ireland	8.8	26	Keegan and Holland (2010)
	Brazil	6.7	57.5	Merigueti et al.(2017)
	Turkey	14	58	Oge et al.(2006)
	Netherlands	12.2	17	Overgaauw et al.(2009)
	Italy	2.9	nd	Paoletti et al.(2015)
	Ireland	67	583.88	Roddie et al.,2008
	Mexico	41.7	nd	Rojas et al.(2017)
	north-west of Iran	36.2	nd	Tavassoli et al.(2012)
	Ireland and Britain	25	71	Wolfe and Wright (2003)

Nd. Not determined.

In conclusion, this study has provided evidence that cats, as in the case of dogs, are capable of carrying *Toxocara* spp. eggs on their hairs. Although soil contamination with *Toxocara* spp. eggs is significantly responsible for human infection, ingestion of eggs through direct contact with cat has been suggested as an alternative route of transmission for this zoonosis. Education of the public about the zoonotic potential of *T.cati*, the prevention of environmental contamination with cats' feces, reduction of the stray cat population and the use of anthelmintics and animal hygiene can help to prevent contamination of human toxocariasis.

Conflicts of interest

The author(s) indicated no conflicts of interest.

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