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### Original Article

## Survey of Dogs' Parasites in Khorasan Razavi Province, Iran

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### Abstract

**Background:** Dog is known to act as definitive host for some parasites that cause important diseases in man and animals. The aim of the present study was to determine the prevalence of *Neospora caninum* and other intestinal parasites in dogs in Khorasan Razavi Province, Iran.

**Methods:** A cross-sectional study was done concerning frequency of *N. caninum* and other intestinal parasites in dogs in Mashhad area. Totally, 174 fecal samples from 89 farm dogs and 85 household dogs were collected from 2006 to 2007. Fecal samples were examined for detecting intestinal parasites by Mini Parasep<sup>®</sup>SF faecal parasite concentrator in Department of Parasitology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran

**Results:** The overall prevalence of other intestinal parasites in farm dogs and household dogs were 29.21% and 14.11%, respectively. Seven parasites were found in farm dogs as follows: *Toxocara canis* 17.9%, *Taenia* sp. 10.1% , *Strongyloides stercoralis* 5.6%, *Hammondia Neospora-like oocysts* (HNLO) 4.4% , *Isospora* sp. 7.8 % , *Sarcocystis* sp. 7.8 % and *Giardia* sp. 1.1% and four parasite in housed dogs: *Toxocara*. 4.4%, *Taenia* sp. 3.3 % , *Isospora* sp. 2.3 % and *Sarcocystis* sp. 4.7 % . The fecal samples with HNLO were examined by *N. caninum* –specific PCR, and two of samples were positive for *N. caninum*.

**Conclusion:** The farm and household dogs are the source of some important zoonotic and non-zoonotic diseases in Iran.

**Keywords:** Prevalence, Intestinal parasites, Dog, Iran

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## Introduction

Dogs are definitive host of some intestinal parasites that cause important diseases in man and animals. Some of parasites are zoonotic agents and important in public health e.g. *Echinococcus granulosus*, *Toxocara canis* and *Giardia intestinalis* (1) and another parasite *Neospora caninum* has been recognized as a major cause of infectious abortion in dairy cattle in the world (2, 3). The introduction of a new dog to a farm with endemic bovine neosporosis appears to be a risk factor for horizontal transmission in herd (4). Thus, in several countries bovine abortion storms were also attributed to horizontal transmission of *N. caninum* (4).

Hydatid cyst and toxocariasis are known zoonotic diseases with high prevalence in Iran (5). Currently, high seroprevalence of *Neospora* infection were reported in dairy cattle (6- 8) and dogs (9, 10). It has been recognized as the most important infectious abortion cause in cattle in Iran (11). All of these parasites are shed eggs, oocysts, and cysts and can be diagnosed by microscopically examination of faeces. In Iran, more studies have been done about intestinal helminthes in order to identify the significance of stray dogs as potential reservoirs of *E. granulosus* (12-16).

The aim of study was to determine the prevalence of intestinal parasites in farm dogs and household dogs and to detect *N. caninum* oocysts infection in farm dogs.

## Materials and Methods

### Field study area

The study was done in Mashhad area, capital city of the Razvi Khorasan Province, situated in the northeast of Iran. The climate is semi-arid with cold winters and moderate summer. The most common breed cattle were Holstein- Friesian. In this

region, the dogs are kept for looking after farm and house.

### Fecal examination

A total of 174 fecal samples from 89 farm dogs and 85 household dogs were collected from 2006 to 2007. Farm dogs were selected from dairy farms that had previously exhibited an abortion problem, and that had participated in a study published earlier (7). Fecal samples of household dogs were collected from dogs presented to the clinic of faculty of veterinary medicine, Ferdowsi University of Mashhad.

Samples were labeled with the names of the owners and kept at cold condition until laboratory examinations took place. Samples were examined by Mini Parasep<sup>®</sup> SF faecal parasite concentrator (Diasys Europe Ltd). Briefly, lid is unscrewed and add 3.3 ml of 10% buffered formalin to the mixing tube and a pea sized (0.4g) fecal sample is introduced by using the spoon on the end of parasep. The sample is mixed in thoroughly with the Parasep spoon. Parasep is immediately sealed by screwing in the filter thimble and conical tube. The mixture is vortexed and Parsep is then inverted to allow the mixture tube filtered through the filter thimble. Parasep is then centrifuged at 3000 rpm for 1 min. The mixing chamber and filter thimble are unscrewed and discarded. All the liquid above the sediment is poured off and added 1 ml water to sediment. The sediment is re-suspended with water by shaking. The sediment then is pipetted to slide for microscopic examination.

All samples from dogs were examined individually for helminthes eggs, coccidian oocysts and other protozoan cysts (17). If the sample had oocysts, the oocysts of faeces were measured with a calibrated ocular micrometer using bright-field microscopy. The oocysts with a diameter of  $11.5 \pm 1.5$  mm and exhibiting morphology similar to non-sporulated *T. gondii*-oocysts were considered

positive for *Hammondia/Neospora*-like oocysts (HNLO) (18-20). HNLO were concentrated and purified from fecal samples by a flotation method using a saturated sucrose-solution (20, 21). Then, an appropriate number of oocysts were used for DNA-isolation. Also, for detecting *Cryptosporidium* oocysts, a smear was prepared from feces and stained by modified acid-fast staining (22)

### Polymerase chain reaction (PCR)

Oocysts of *N. caninum* are morphologically indistinguishable from *Hammondia heydorni* and *Toxoplasma gondii* (21). It was necessary to do molecular methods such as PCR for differentiating oocysts of *N. caninum* from *H. heydorni* and *T. gondii*. The procedure for *N. caninum*-specific PCR was carried out as described earlier by using the primer pair Np6<sup>+</sup>/Np21<sup>+</sup> (23, 24). First, the oocysts were ruptured by two to three freeze-thaw cycles. Then, DNA was subsequently isolated from purified oocysts with the DNeasy-kit according to the manufacturer's instructions (Cina-gen, Iran). After that, DNA of amplification was performed above method with *Neospora* specific primers: Np6<sup>+</sup> (5'-CTCGCCAGTCAACCTACGTCTTCT-3'), and Np21<sup>+</sup> (5'-CCCAGTGCGTCCAATCCTGTAAC-3').

### Statistical analysis

Data were analyzed by Chi-square test. Values of  $P < 0.05$  were assumed significant (25).

### Results

The overall prevalence of intestinal parasites in farm dogs and household dogs were 29.21% and 14.11%, respectively (Table.1) ( $P < 0.05$ ). Monospecific infestation were found in 14.9% of dog whereas concurrent infestation with 2 or more species in 7.47% (Table 1). Seven and four parasites were found in farm and household dogs, respectively (Table.2) ( $P < 0.05$ ). Seven parasites

were found in farm dogs as follows: *Toxocara canis* 17.9%, *Taenia* sp. 10.1%, *Strongyloides stercoralis* 5.6%, *Hammondia Neospora-like* oocysts (HNLO) 4.4%, *Isospora* sp. 7.8%, *Sarcocystis* sp. 7.8% and *Giardia* sp. 1.1% and four parasites in household dogs as follow: *Toxocara canis* 4.4%, *Taenia* sp. 3.3%, *Isospora* sp. 2.3% and *Sarcocystis* sp. 4.7%. (Table 2) ( $P < 0.05$ ). The oocyst per gram of HNLO in examined fecal samples was low (5-10 oocysts per gram). Four samples with HNLO were tested by *N. caninum*-specific PCR. Two samples were positive for *N. caninum*. One farm dog was male and 2 years old and the other was male and 4 months old.

### Discussion

In this study, the overall prevalence of intestinal helminthes were very lower than the results of previous studies in Iran (26-28). These results can be easily explained, because, all of previous studies were done in stray dogs that have no health control measure. The higher prevalence was observed in farm dogs in compared to household dogs. This result also was expected, because the farm dogs had bad hygienic conditions, was fed by uncooked meats or offal, and used less antihelminthic drugs. *T. canis* and *Taenia* sp. were the most commonly encountered parasites in two populations of dogs. The probability of *Echinococcus* infestation in every positive dog with *Taenia* sp. is relatively high, therefore, it needs to educate dog owner about the mode of transmission and prevention methods for control of hydatid cyst and visceral larval migraine.

*S. stercoralis* was only observed in farm dogs. Its prevalence was higher than other studies in Iran (16, 28). It seems that detection of eggs or larvae L1 of *S. stercoralis* in coprological examinations are easier than finding mature helminths with small size in postmortem examination. However, *Strongyloides* infections in farm dogs are considered as a potential public health hazard.

**Table1:** Frequency of single and multiple intestinal parasites in farm dogs and household dogs in Mashhad area.-Iran

	Farm dog No. (%)	Housed dog No. (%)	Total No. (%)
<i>Hammondia Neospora</i> -like oocysts (HNIO)	1(1.1)	0 (0)	1(0.57)
<i>Toxocara canis</i>	7(7.8)	3(3.5)	10(5.7)
<i>Taenia</i> sp.	5(5.6)	2(2.3)	7(4.02)
<i>Sarcocystis</i> sp.	1(1.1)	4(4.7)	5(2.8)
<i>Isoospora</i> sp.	1(1.1)	2(2.3)	3(1.72)
<i>Toxocara canis</i> and <i>Taenia</i> sp.	2(2.2)	1(1.1)	3(1.72)
<i>Isoospora</i> sp. and <i>Toxocara canis</i>	1(1.1)	0	1(0.57)
<i>Toxocara canis</i> and <i>Taenia</i> sp. and <i>Hammondia Neospora</i> -like oocysts (HNIO)	2(2.2)	0	2(1.1)
<i>Isoospora</i> sp. and <i>Sarcocystis</i> sp. and <i>Strongyloides stercoralis</i>	1(1.1)	0	1(0.57)
<i>Sarcocystis</i> sp. and <i>Giardia</i> sp. <i>Hammondia Neospora</i> -like oocysts (HNIO)	1(1.1)	0	1(1.1)
<i>Isoospora</i> sp. and <i>Sarcocystis</i> sp. and <i>Strongyloides stercoralis</i> and <i>Toxocara canis</i>	4(4.4)	0	4(2.2)
Total	26 (29.21)	12(14.11)	38 (21.81)

**Table2:** Frequency of intestinal parasites in 89 farm dogs and 85 housed dogs in Mashhad area.-Iran

Parasite	Helminths No. (%)			Total	Protozoa No. (%)				Total
	<i>Toxocara canis</i>	<i>Taenia</i> sp.	<i>S. stercoralis</i>		HNIO*	<i>Giardia</i> sp.	<i>Isoospora</i> sp.	<i>Sarcocystis</i> sp.	
Farm dog	16 (17.9)	9 (10.1)	5 (5.6)	30 (33.70)	4 (4.4)	1 (1.12)	7 (7.8)	7 (7.8)	19 (21.34)
Housedog	4 (4.7)	3 (3.3)	0	7 (8.2)	0	0	2(2.3)	4 (4.7)	6 (7.05 )
Total	20 (11.49)	12 (6.8)	5 (2.8)	37 (21.26)	4 (2.2)	1 (0.57)	9 (5.1)	11 (6.32)	25 (14.36)

\*HNIO: *Hammondia Neospora*-like oocysts

In the present study, *Cryptosporidium* sp. was not microscopically detected and *Giardia* sp. was only observed in one sample of farm dog. The prevalence of *Giardia* sp. was lower than two previous studies (29, 30) and similar to one study that done in Iran (31). The prevalence of *Giardia* sp. varies widely depending on the geographic locality, detection methods and population understudied. The parasites such as *Giardia* sp. and *Cryptosporidium* sp. can be difficult to detect using conventional microscopy and need to sensitive methods such as PCR.

*Isospora* sp. and *Sarcocystis* sp. were found in two populations of dogs. Although, a few epidemiologic studies were done about *Sarcocystis* sp., *Isospora* sp. in Iran (29, 31), but, comparison of results was shown that *Isospora* sp. and *Sarcocystis* sp. are very prevalent among different population of dogs in Iran and needs more investigations.

HNLOs were only found in four samples of farm dogs, because, dogs excrete *N. caninum* oocysts after eating placentas of naturally infected cattle and tissues of experimentally infected calves (32-34). Therefore, it is important to detect properly the *N. caninum* oocysts in feces samples. In the present study, DNA of *Neospora* was only confirmed in two fecal samples by *N. caninum*-specific PCR. Because, the presence DNA of *Neospora* in samples may be due to the feeding of infected of fresh and uncooked meat, there is a doubt about presence of *N. caninum* oocysts in fecal samples. For confirmation, it needs to isolate the parasites in gerbils' bioassay or cell culture.

Conclusively, the results of this study indicate that the main risks to public health are *T. canis* and *Taenia* sp. responsible for the production of larva migrans syndromes and or hydatid cyst in man who meet infecting larvae or eggs of helminths. Other zoonotic parasites such as *S. stercoralis* and *Giardia* sp. in very low prevalence have been detected in farm dogs. Among non-zoonotic intestinal parasites, HNLO samples were only detected in farm dogs that may be the most important

source of *N. caninum* infection in dairy cattle in Iran.

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