

## Enteric protozoa of dogs: prevalence, associated risk factors and owners' awareness in and around Hawassa town, Ethiopia

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### Abstract

Dogs are reservoirs of zoonotic pathogens and pose a significant health risk to humans in most parts of the world. A cross sectional study design was used to estimate the prevalence of enteric protozoal parasites in dogs, to assess the associated risk factors and the level of owners' awareness about zoonotic implications of enteric protozoa parasites in selected subcities of Hawassa town from October 2015 to June 2016. A total of 340 faecal samples were collected and examined for enteric protozoa using flotation techniques. Of these, 171 (50.3%) dogs were infected with one or more genera of enteric protozoa parasites. The parasites identified were *Sarcocyst* (28.5%), *Isospora* (20.6%), *Cryptosporidium* (8.5%), *Neospora* (8.2%) and *Giardia* (5.6%). About 34.5% of the examined dogs were infected with two to four enteric protozoa parasites. The overall prevalence of enteric protozoa parasites was significantly higher in younger dogs ( $\chi^2= 20.21, p < 0.05$ ), semi-confined ( $\chi^2= 5.63, p < 0.05$ ) and stray dogs ( $\chi^2= 23.01, p < 0.05$ ). The prevalence of *Sarcocyst* ( $\chi^2= 14.45, p < 0.05$ ), *Isospora* ( $\chi^2= 4.38, p < 0.05$ ) and *Neospora* ( $\chi^2= 4.31, p < 0.05$ ) was significantly higher in stray dogs as compared to owned dogs. Most of respondents and dog owners' (76.5%), had no awareness on zoonotic enteric protozoa parasites of dogs. But 32.8% and 23.2% the respondents' children hug and play with dogs, respectively. Most of them (71.7%) feed their dogs with uncooked butcher leftover and/or offals. About 66.2% of them dispose of dogs' feces with garbage. Therefore, extension works for public education to improve public awareness on parasitic zoonoses, is very helpful to reduce the problem to the possible minimum level. Moreover, a close collaboration between veterinarians and public health professionals is highly helpful.

**Keywords:** Dogs; Enteric protozoa; Ethiopia; Hawassa; Risk factors; Zoonotic

## Introduction

Dogs are becoming an important part of societies throughout the world. They have probably the closest contact with human, and could act as reservoirs or carriers and transmitters of zoonotic diseases (Robertson *et al.*, 2000; Irwin (2002). Schantz (1994) reported that the potential health risk to humans of enteric parasites harbored by dogs remains a major problem in most parts of the world. Although none of zoonotic enteric protozoal diseases are life-threatening to humans, several of them can lead to severe illness and are therefore a public health concern. The risk of severe illness is particularly high for the most vulnerable group of the society, namely children, the elderly, pregnant women and immunocompromised (Irwin, 2002). Most of the enteric protozoa of dogs can be transmitted to the domestic livestock in a various ways of transmission mechanism (Dubey *et al.*, 2007; Taylor *et al.*, 2007)

In humans, cryptosporidiosis and giardiasis are both important emerging and “re-emerging diseases” (Martínez-Moreno *et al.*, 2007). These parasites are considered to be a significant risk to immunocompromised people and are commonly recognized causes of diarrhea in infant day care centers (WHO 1996; Robertson *et al.*, 2000). Significant clinical infection has also been reported in immunocompetent adults (Martins and Guerrant, 1995; Ramirez-Barrios *et al.*, 2004).

*Giardia* is common in dogs and cats and in some occasions it is associated with overt symptoms or clinical disease. More importantly, dogs and cats can carry strains of *Giardia* which are potentially infective to humans (Hopkins *et al.*, 1997). *Giardia* species are a frequent cause of diarrhea in immunocompromised people including children, if left untreated, may cause persistent infection leading to irregular episodes of gastrointestinal illness (Ochoa *et al.*, 2004).

*Cryptosporidium* has been widely detected in dogs and cats (Milstein and Goldsmid, 1995; Sargent *et al.*, 1998) and these animals may represent an important reservoir of infection for humans. The infective oocyst may be transmitted to human directly by the fecal/oral route, or through contamination of water supplies (Robertson *et al.*, 2000). It is a common cause of human diarrhea (Current and Garcia, 1991; Hunter and Nichols, 2002). The number of detected human cases began to rise rapidly alongside the AIDS pandemic (Avery *et al.*, 2007). There is a growing concern about the zoonotic potential of *Neospora*

*caninum*. However, at present there is no firm evidence that *N. caninum* successfully infect humans. But it is transmitted to cattle and causes abortion (Dubey *et al.*, 2007).

Most of the intestinal protozoan infections do little or no harm to dogs, especially in adult dogs, these infections go unnoticed or neglected (ESCCAP, 2011). The importance of zoonotic parasites in dogs is evident in most parts of the world. But there is scarcity of information and there is very rare or no research work on zoonotic protozoa parasites of dogs in Ethiopia. Moreover, there is lack of awareness among dog owners about the common zoonotic parasites that could be carried by their dogs and their mode of transmission. There is an increasing number of companion animals, especially dogs, kept in close interaction with human beings in Hawassa town. But there is no information available on the status of enteric protozoa. Therefore, the objectives of this study was to estimate the prevalence of dog enteric protozoa, to assess the potential risk factors associated with the occurrence of the problem and to assess the level of owners' awareness about the enteric protozoa parasites of dogs that are zoonotic in Hawassa town.

## Materials and methods

### Study area

The study was conducted in and around Hawassa town, capital of Southern Nation Nationalities People Regional State (SNNPRS), from October 2015 to June 2016. It has an area of 162,804 hectares and about 399,461 human populations (CSA, 2013). Hawassa is located between 4°27' and 8°30' N latitude, and 34°21' and 39°1'E longitude at an altitude of 1790 m above sea level. The study area is characterized by short (February to April) and long (July to October) rainy seasons. The mean annual rainfall is 1091mm, and the mean annual minimum and maximum temperature are 13°C and 27°C, respectively (National Meteorological Agency, 2016).

### Study animals

The study animals were dogs found in and around Hawassa town. Dogs of different age groups and both sexes were selected by systematic random sampling technique from confined, semi-confined (roaming at night only) management system and stray dogs, free roaming all the time. For simplicity, dogs up to one

year of age were grouped under young age group, while those between 1 and 3 years as adult and those older than 3 years as old. From eight subcities, based on the convenience, willingness and cooperation of owners two sub-cities, Tabor and Meneharia, were included in the study.

## Study design and sampling

A cross-sectional study design was employed to estimate the prevalence of enteric protozoa parasites, and to assess the level of dog owners awareness of zoonotic enteric protozoa parasites. The study was conducted using active laboratory investigation and questionnaire survey. Age, sex, and management system of dogs were recorded through personal communication with the dogs owners during faecal sample collection. The sample size was determined following the formula described by Thrusfield (2005), and 20% expected prevalence (Pfeiffer, 2002). The study considered 5% absolute precision with 95% confidence interval. As a result, the sample size computed was 246; and however, an additional 94 animals were included and a total of 340 were examined.

During fresh faecal sample collection, the color and consistency of the feces were recorded. Collected faecal samples were placed in clean and sterile universal bottles, labelled and immediately transported to Parasitology laboratory of Hawassa University, School of Veterinary Medicine. Then, it was examined by flotation technique using 33% Zinc sulfate solution with specific gravity of 1.2 (Zajac and Conboy, 2012). Faecal sample was examined on the date of collection, and if not it was preserved with 10% formalin and examined within two days of the collection. The oocysts and/or trophozoites were identified based on their morphological characteristics as described by Hendrix (1998) and MAFF (1977). For detection of *Cryptosporidium*, the faecal samples were treated with formol-ether concentration and stained by modified Ziehl-Neelsen technique as described by Henriksen and Pohlenz (1981).

## Questionnaire survey

A pre-tested semi-structured questionnaire was used to collect information about dogs' housing management and cleaning practices (i.e. use of kennel, cleaning and dog waste disposal), feed and feeding (i.e. major feed source for dog and habit of meat cooking for dog), awareness of dogs' zoonotic parasites

and children-dog interaction (i.e. whether not approach to dogs, play with dogs, and hug and play with dogs).

### Data management and analysis

Data collected were entered into Microsoft Excel spreadsheet, edited and coded. Descriptive statistics was used to summarize the data. Chi-square test and logistic regression analysis were used to assess differencene in prevalence between the various risk factors that considered for this study. For the data analysis STATA software, window version 13.1 (StataCorp 4905 Lakeway DriveCollege Station, 2013) was used. The study considered 95% confidence level and 5% desired level of precision.

## Results

### Overall prevalence of enteric protozoa parasites

Of the total 340 dogs examined 171 (50.3%) of them were found infected by enteric protozoa parasites. Five genera of enteric protozoa parasites were identified namely: *Sarcocyst*, *Isospora*, *Cryptosporidium*, *Neospora* and *Giardia* (Table 1).

**Table 1. Prevalence of enteric protozoa parasites of dogs in Hawassa (n = 340).**

Protozoa genera	No of positive dogs	Prevalence (%)	95% CI
<i>Isospora</i> spp.	70	20.6	16.3–24.9
<i>Cryptosporidium</i> spp.	29	8.5	5.5–11.5
<i>Neospora</i> spp.	28	8.2	5.3–11.2
<i>Sarcocysts</i> spp.	97	28.5	23.7–33.4
<i>Giardia</i> spp.	19	5.6	3.1–8.0
Overall	171	50.3	44.9–55.6

From 171 infected dogs about 59 (34.1%) were found to harbor two to four genera of enteric protozoa parasites (Table 2). About half of the examined dogs were found harboring one or more than one types of enteric protozoa. From the total infected dogs fifty nine of them (34.5%) were infected with more than one genus of the identified enteric protozoa.

**Table 2. Proportion of dogs infected with single and multiple genera of enteric protozoa (n=171)**

Number of species	Frequency	Prevalence %	95% CI
One	112	32.9	28.1-38.1
Two	48	14.1	10.8-18.3
Three	9	2.6	1.4-5.0
Four	2	0.6	0.1-2.3

**Risk factors for enteric protozoa parasites infection**

The analysis results for the risk factors considered in the study are shown in Table 3. The prevalence of enteric protozoa was significantly higher in younger ( $\chi^2=20.21$ ,  $p < 0.05$ ) and free roaming ( $\chi^2= 23.32$ ,  $p < 0.05$ ) dogs.

**Table 3. Prevalence of enteric protozoa in dogs and the associated risk factors.**

Risk factors and levels	No. examined	Prevalence (%)	95%CI	$\chi^2$	p-value
<b>Sex</b>					
Male	229	54.1	47.7- 60.6		
Female	111	44.1	34.8 -53.5	2.99	0.084
<b>Age group</b>					
≤1 year	86	66.3	56.2-76.4		
1 to 3 years	118	55.9	46.9 -65.0		
Over 3years	136	36.8	28.6 -44.9	20.21	<0.001
<b>Village</b>					
Tabor	181	52.8	40.7-55.4		
Meneharia	159	48.1	45.0-60.6	0.768	0.381
<b>Management</b>					
Free roaming	93	69.9	60.5-79.3		
Semi-confined	150	49.3	41.3-57.4		
Strictly confined	97	35	25.5-44.6	23.32	<0.001

The prevalence of the identified enteric protozoa parasites among the management system of dogs is shown below (Table 4). The overall enteric protozoa parasites prevalence significantly varied among the three management systems. It was higher in semi-confined ( $\chi^2= 5.63$ ,  $p < 0.05$ ) than strictly-confined

dogs; and it was significantly higher in stray ( $\chi^2= 8.88, p < 0.05$ ) than in semi-confined dogs.

**Table 4. Linear logistic regression analysis of dogs' management system and enteric protozoa prevalence.**

Protozoa	Management system	No. examined (No. positive)	Prevalence % (95% CI)	OR	Z	P-value
<b>Isospora species</b>	Strictly confined	97 (15)	15.5(8.2-22.7)	-		
	- Semi-confined	150 (29)	19.3(13.0-25.7)	1.31	0.77	0.438
	- Stray	93 (26)	20.0(18.8-37.2)	2.12	2.07	0.039
<b>Cryptosporidium spp.</b>	Strictly confined	97 (5)	5.2(0.7-9.6)	-		
	- Semi-confined	150 (16)	10.7(5.7-15.6)	2.20	1.49	0.137
	- Stray	93 (8)	8.6(2.9-14.4)	1.73	0.93	0.352
<b>Sarcocyst species</b>	Strictly confined	97 (18)	18.6(10.8-26.4)	-		
	- Semi-confined	150 (38)	25.3(18.3-32.3)	1.49	1.24	0.216
	- Stray	93 (41)	44.1(33.9-54.3)	3.46	3.71	<0.001
<b>Neospora species</b>	Strictly confined	97 (5)	5.2(10.8-26.4)	-		
	- Semi-confined	150 (10)	6.7(18.3-32.3)	1.31	0.48	0.628
	- Stray	93 (13)	14.0(33.9-54.3)	2.99	2.00	0.046
<b>Giardia species</b>	Strictly confined	97 (3)	3.1(0.4-6.6)	-		
	- Semi-confined	150 (8)	5.3(1.7-9.0)	1.77	0.82	0.410
	- Stray	93 (8)	8.6(2.9-14.4)	2.95	1.56	0.119
<b>Overall enteroprotezoa</b>	Strictly confined	97 (33)	35.1(24.5-43.5)	-	-	
	- Semi-confined	150 (74)	49.3(41.3-57.4)	1.90	2.26	0.024
	- Stray	93 (64)	69.9(59.3-78.3)	6.18	5.21	<0.001

The prevalence of *Giardia* spp., *Cryptosporidium* spp. and *Isospora* spp. were significantly higher in dogs with diarrheic feces (Table 5).

**Table 5. Enteric protozoa prevalence with respect to fecal consistency of dogs**

Feces consistency	No of dogs infected (%)			
	<i>Giardia</i> spp.	<i>Cryptosporidium</i> spp.	<i>Isospora</i> spp.	Overall protozoa
Diarrheic (61)	8(13.1)	14(23.0)	19(31.1)	54(88.5)
Non-diarrheic(279)	11(3.9)	15(5.4)	51(18.3)	119 (42.7)
$\chi^2$	7.98	19.81	5.07	42.15
<i>P-value</i>	0.005	<0.001	0.024	<0.001

### Dog owners' awareness of zoonotic enteric protozoa

The questionnaire survey about owners' awareness of zoonotic enteric protozoa of dogs revealed that 76.5% had no awareness about zoonotic parasites. The result of dog owners' awareness and dogs management system are shown in Table 6.

**Table 6. Summary of dogs management and owner's awareness about zoonotic protozoa (n=293)**

Variables	Factors level	Frequency	Percentage
<b>Management</b>			
<b>Use of kennel</b>	Yes	254	83.83
	No	49	16.17
<b>Kennel cleaning</b>	Yes	246	96.85
	No	8	3.15
<b>Frequency of cleaning</b>	Once per month	40	16.26
	Once per week	37	15.04
	Daily	140	56.91
	Following defecation	29	11.79
<b>Dogs waste disposal</b>	Outside the compound	49	17.07
	On garden area	9	3.66
	Taken by garbage collectors	163	66.2
	Buried	2	0.81
	Dumped in toilet	30	12.20
<b>Feed and Feeding</b>			
<b>Major food source</b>	Family leftover	112	38.2
	Butcher leftover	150	52.2
	Leftover from hotel	7	2.4
	Left free to scavenge	20	6.8
	Dairy byproduct	4	1.4
<b>Cooking meat for dog</b>	Yes	83	28.3
	No	210	71.7
<b>Awareness on Zoonoses</b>	Yes	188	64.2
	No	105	35.8
<b>Measure on sick dog</b>	Treat with available drug	62	21.2
	Call a Vet/Visit Vet clinic	191	65.2
	Abandon the dog	8	2.7
	Do nothing	32	10.9
<b>Children -Dog interaction</b>	Not approach to dog	129	44
	Play with dogs	96	32.8
	Hug and play with dogs	68	23.2

## Discussion

This study revealed an overall enteric protozoa prevalence of 50.9% in dogs in and around Hawassa, Ethiopia. This is relatively high level of infection; and comparable to the report of Adejinmi and Osayomi (2010). This finding is higher than the reports from various areas: Martinez-Moreno *et al* (2007), Gingrich *et al* (2010), Mahmud *et al* (2014) and Mirzaei (2010) who reported 12.11%, 13.4%, 21.7% and 13.26% from Spain, Galapagos Islands of Ecuador, Bangladesh and Iran, respectively. Such differences in the prevalence of enteric protozoa could be due to variation in geographic location, owners' awareness and the number of stray dogs' population in an area. The enteric protozoa identified during this study were *Cryptosporidium* spp. (8.5%), *Giardia* spp. (5.6%), *Isospora* spp. (20.6%), *Neospora* spp. (8.2%), and *Sarcocysts* spp. (28.5%). This observation is in agreement with reports from various areas (Khalaf *et al.*, 2015; Ramirez-Barrios *et al.*, 2004; Benito *et al.*, 2003). Dogs harbouring a single enteric protozoa parasite species were more common (32.9%) than those harbouring 2 (14.1%) or multiple species. The finding of infections with more than one enteric protozoa parasites is not surprising, and similar findings have been reported by various authors (Alvarado-Esquivel *et al.*, 2015; Bahrami *et al.*, 2011; Cantó *et al.*, 2011; Adejinmi and Osayomi, 2010; Gingrich *et al.*, 2010; Mukaratirwa and Singh, 2010; Gracenea *et al.*, 2009; Nikolic *et al.*, 2008). The prevalence of enteric protozoa parasites of dogs were significantly higher in free roaming and younger dogs. This finding is in agreement with Symeonidou *et al* (2017), Awadallah *et al* (2015), Adejinmi and Osayomi (2010) and Mirzaei (2010) who reported higher prevalence of protozoa parasites in younger dogs.

The overall prevalence of enteric protozoa was significantly higher in stray and semi-confined than in strictly confined dogs. But the prevalence of *Isospora* spp., *Sarcocyst* spp. and *Neospora* spp. were significantly higher in stray dogs than strictly-confined dogs. *Sarcocyst* spp. is the only enteric protozoa that significantly varied among the management system of dogs in the study area. The possible justification for this higher prevalence in stray dogs could be due to their frequent exposure to infected animals tissues, absence of treatment, and higher access to rodents. Moreover, they had free access to visceral organs, aborted foetus and placenta of intermediate host animals (Sager *et al.*, 2006). These dogs may be at risk of acquiring infections with cyst-forming coccidian like *Neospora* spp. and *Sarcocystis* spp. (ESCCAP, 2011).

The overall prevalence of enteric protozoa parasites were significantly higher in diarrheic dogs. Also *Giardia* spp., *Isospora* spp. and *Cryptosporidium* spp. were similarly more prevalent in diarrheic dogs. Dogs infected of by *Giardia* spp. and *Cryptosporidium* spp. may be clinically manifested by pasty to watery diarrhea (ESCCAP, 2011; Taylor *et al.*, 2007).

*Giardia* species detected (5.6%) during this study was lower than the reports from different parts of the world (Alvarado-Esquivel *et al.*, 2015; Mahmud *et al.*, 2014; Li *et al.*, 2012; Bahrami *et al.*, 2011 and Nikolic *et al.*, 2008). This could be due to the intermittent excretion of *Giardia* cysts, which lasts for few days (Villeneuve *et al.*, 2015). So, it is suggested to take samples and examine three times over 3-5 days. Generally, the prevalence of *Giardia* varied from 10% to 100% depending on the sensitivity of the test employed, age of the examined dogs and the level of care and management of dogs (Hahn *et al.*, 1988; Thompson *et al.*, 2008).

During this study we found that 52.2% and 38.2% of the dog owners fed their dogs with families and butcher leftover, respectively. Most of them (71.7%) fed their dogs uncooked butcher leftover and/or offals. This finding is in agreement with the report of Amisshah-Reynold *et al* (2016). Almost one-third of the respondents had no awareness about zoonosis, and their children play with their dogs. According to the information collected from the respondents, the most common means (66.2%) of dogs' feces disposal was with garbage. This finding is in agreement with Njong *et al* (2012). This study showed that, the awareness of dog owners on dogs' management, feeding and zoonosis is poor. Moreover, dog owners' children having close contact with their dogs and improper feces disposal, taken by garbage collectors, can threaten the public health.

## Conclusion

The study revealed that about half of the dogs examined (50.3%) were found infected with one or more enteric protozoa parasites. The enteric protozoa parasites detected during this study were *Cryptosporidium*, *Giardia*, *Isospora*, *Sarcocyst* and *Neospora* species. Most of them are zoonotic, posing risk for those people living in the study area. It is particularly important in children playing with dogs. Awareness of dog owners about zoonosis, and as a result feeding of their dogs with uncooked butcher leftover might have contributed a lot for their transmission. Therefore, proper disposal of feces, cooking butcher leftover and keeping children away from dogs should be practiced to prevent

their spread and transmission. In addition, creation of public awareness about zoonotic parasites play key role. Moreover, a close collaboration between veterinarians and public health professionals is highly helpful.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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