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Prevalence of intestinal protozoans parasites of household dogs and risk perception of their Zoonosis in District Swabi, Pakistan

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Dogs play various roles and their presence within people's houses has increased. In rural and semi-rural and in urban settings dog feaces are not removed from the streets representing an environmental pollution factor. The aim of current study was to evaluate the occurrence of environmental contamination with protozoal intestinal parasites of five breeds of dogs in District Swabi, Pakistan. We collected 128 dog faecal samples from district Swabi, Pakistan. We isolated upto five different parasites in a single sample and detected nine helminth and three protozoans' parasite species in combination. The intestinal helminths detected were *Ascaris lumbricoides* 31.2% (n=15), *Taenia spp* 25% (n=12), *Toxocara canis* 18.7 (n=9), *Diphylidium caninum* 8.3% (n = 4) Toxascaris 2.0% (n=1), *Ancylostoma caninum* 6.02% (n=3), *Trichuris vulpis* 2.0% (n=1), *Echinococcus sp 2.0*% (n=1)., *Strongyloides stercoralis 4.1% (n=2),.* Amongst the protozoans *Giardia spp* 30.7% (n=8), *Coccidia spp* 42.3% (n=11), and *Isospora spp* 26.9% (n=7). Pattern of infection revealed that 9 (7.03%) double, 7(5.40%) triple, 7 (5.40%) quadruple and 3 (2.30%) pentruple infection. The mixed breeds were highly infected 27.2% while the lowest 17.0% (n=21) puppy dogs were recorded. Significant difference was observed in the prevalence of infection with intestinal parasites among these breeds (P >0.05). The result of current study highlight a severe environmental contamination by frequent parasitic stages infectious to humans. There is a higher risk of zoonotic transmission from dogs which indicate an immediate need for the controlling of these parasites and educating the public to take wise action relating to the parasites and pets.

Keywords: gastrointestinal parasites, environmental parasitic contamination, Zoonosis, canids, Pakistan

INTRODUCTION

Dog faeces denote a main pollution source particularly in rural and semi-rural areas which are not generally removed on routine basis. Likewise, vehicular road traffic, as well as the wind, can help spread potential pathogens present in dog faeces, polluting food which might future be a cause of infection (Smith, 1995). Moreover, Insects and other environmental factor, such as the rain, wind may also play an important role in this perspective. To reduce and lighten the load of zoonotic illness on public health it is necessary to monitor dogs on regular basis for zoonotic gastrointestinal parasites for control and prevention strategies. Though, there is no report regarding occurrence of gastrointestinal parasites

from dogs in district Swabi, which is a missing link. To fulfil this gap of study in our area to coincide it to worldwide knowledge, we design and plane study to estimate the prevalence of GI parasites in owned dogs in Swabi District.

In developing countries risk factors of zoonotic infection related to house hold dogs is high due to no restriction on dog owners as well as having no sense of responsibilities among dog's keepers (Macpherson, 2005). Domestic dogs harbour parasites of great zoonotic importance. Dogs' human interaction lead a potential risk, particularly in children and patients having less immunity (Robertson *et al.,* 2000).

In present study area there is no proper management of dogs to be registered officially, so the actual extent of dog's population is unidentified. Information regarding present study about area prevalence is important for the development and modification of control measures in animal and public health (Wang and Tedford, 2007). The consensual report is evident from the countries surround the Mediterranean Sea that there is deficiency of data on the frequency of zoonotic diseases in wildlife faunas and in public, which avoids a current epidemiological investigation and leads to the failure in stoppage. Also, there is no co-ordination, communication, between public and health sectors, as well as lack of collaborated work between human and animal health professionals, along with no knowledge of the human population. Importantly, a quite small number of reports have focused on the infection risk of inhabitants living in rural or suburban locations (Robertson, and Thompson, 2002) where commonly dogs defecate outside towns and cities (Fisher, 2003.) Several rural and semi-rural societies have large populations of free wandering domestic dogs and slight contact to veterinary treatment. However, these dogs have regular interaction with other animals, their faeces, and a variety of waste and products that potentially contain zoonotic representatives, which encourages infection with a variety of animals and following human contact (Palmer, et al. 2008).

Domestic dogs have got comparatively less attention for treatment in developing countries, especially in (Swabi District), and less caring attitude of communities towards them. This general carelessness and other social and economic problems leave the dogs at higher risk of getting infected with parasites and also have the potential to infect humans. Being at the top of food chain dogs and cats play a crucial role in ecosystem maintenance and are also related to humans in a relation as close as no other animal has. So, studying these animals and the risks they are exposed to is very necessary for their as well as our well-being.

Of the risks associated with being in close relationship with pet dogs, the risk of getting infectious diseases is of a larger concern. Human is exposed to certain parasites through these animals including many that cause severe health issue.

Lack of awareness of the owners of the animals, not understanding and fulfilling their responsibilities, lack of sufficient and adequate knowledge with veterinarians, negligence about the need of anti-parasitic drugs are the factors that cause or at least assist in prevalence and transmission of parasites in the animals and also in humans. A large number of studies on gastro-intestinal parasites of dogs have been conducted in various parts of the world including Pakistan. Information on these infections in dog hosts in Pakistanis scarce. This study was aimed to provide information about the prevalence of gastrointestinal parasites of five breeds (Fighter, hunter, German shepherd, Puppy, and Mix breed) of dogs in District Swabi, Pakistan.

MATERIALS AND METHODS

3.1 Study Area

The study area for this research work is district Swabi, Khyber Pakhtunkhwa Pakistan. District Swabi is located at 34°70 N and 72°28 E. The total area of District Swabi is about 1543 km^2 It is located on North West side of Punjab province, at South side it joined with Kamra and attock, West side share boundary with district Mardan, West south is district Nowshehra and North side is Kupri Amazi. The district contains four Tehsils Lahore, Rajor, Topi and Swabi and the residents are referred to as, swabiwall. The map is shown in figure 3.1.

3.2 Area demography

Most of the studies performed in Pakistan regarding GI parasites of dogs have conducted in very limited parts of the country. The current locality (district swabi) was selected for this study because it lacks this type of study and, also, because it is a mainly rural semi-rural and urban one. The municipality belongs to the District of swabi and is located in the Khyber Pakhtunkhwa province (Figure 1). The main source of income in this municipality is agriculture in the rural semi-

rural area, producing mostly corn, potatoes, tobacco, vegetable, while urban areas is congested, having different occupations by the residents.

Figure1: Location of study area District Swabi (highlighted within the Khyber Pakhtunkhwa province of Pakistan.

3.4 Study Design

Faecal samples of `128 dogs (hunter 17, fighter 47, German shepherd 21, puppy 21 and mixed breed 22) were collected and analyse in the parasitological examination. All the animals studied were positioned based on their age, sex and locality. Due to general lack of interest and carelessness about dogs in the community there seldom are pets in the study area so the study was limited to the studied breeds. They often contact with humans and so we have to take samples indirectly. Because of the carelessness of the people the dogs in the area are often scavengers. They rarely have regular food supply, so they eat the optional food. The dogs often live near the residence of their owners.

3.5 Data Collection and Processing

Between June 2017 and November 2018, we collected dog faeces from five different breeds (hunter 17, fighter 47, German shepherd 21, puppy 21 and mixed breed 22) of domestic dogs (Figure 2). Stool samples were collected from the dogs in 24 main town and local places of this municipality. Samples were randomly collected from selected areas in District Swabi mention formerly. Whenever possible, faecal samples were collected immediately after spontaneous elimination. Faecal samples were collected from walking areas of the animals often around human populations. All the samples were fresh and were of maximum a few hours. The collected samples were kept in sterile plastic bottles and 10% formalin was added to it just enough to dip the faeces. After then all samples were individually identified, through coprological methods. All the samples were brought to Laboratory of Parasitology, department of Zoology, University of Malakand. Some of the samples were studied at Department of Zoology, University of Swabi, for parasitological assessment.

3.6 Laboratory examination

The samples were first of all checked with naked eye for any adult visible stage of the parasites and then these were prepared for microscopic examination. Small portion of the sample was taken, and a drop of liquid was added to it. For examining any helminth eggs, and protozoan cysts normal sodium chloride solution (Nacl) was used. The slide was covered with cover slip and examined under microscope. For fixing the slides glycerine was also added to the slide before covering it with cover slip. The specimens were first checked with low power lens (10X) and then with high power lens (40X). This is called wet mount technique (Proudman and Edwards, 1992), which is fruitful for helminths' parasites eggs and protozoan cysts and oocysts. Ova were identified based on their differential morphological features i.e shape, colour, size, ovum, embryo, and also for protozoan parasites examination. All the parasites found were checked with relevant literature and confirmed with the existing morphological keys. A faecal sample was considered infected if at least one helminth egg, oocyst, or cyst was observed.

RESULTS AND DISCUSSION

Of the total dogs infected 7.03% were infected with 2 species, 5.40% with three and four species each where as 3.20% of dogs were found infected with five species of parasites (Table 1).

Of the total 12 species, 9 were nematodes and 3 were protozoans detected in association with each other (Table 2).

In total, N=128 dogs of different breeds were included in this study. Among them 17% were fighter, 17.6% were hunters, 19% were German shepherds, 23.8% puppy and 27.2% were mixed breed (table 3). Overall prevalence of gastro-intestinal parasitic infection among these dogs was determined to be 20.3%. The prevalence of infection with intestinal parasites was significantly different among these five breeds ($P < 0.05$). (Table 3) Males were found to have a lower percentage of infection (18%) compared to the female group (28.5%). The association between the gender and parasitic infection was statistically non-significant ($P > 0.05$). Regarding ages below 1 year were slightly more infected (30%) than above 1 year of age (18.5%). The association between the ages and parasitic infection was also statistically non-significant ($P > 0.05$). Out of the 128 dogs examined, no single dogs were found infected with a single species of protozoans while all the protozoans detected were with other helminths in poly parasitic association. (Table 4).

The current study provides information regarding prevalence of gastro-intestinal parasitic infection in various breeds of dogs from district Swabi, Pakistan. These parasites remain the most common intestinal parasitic pathogens in the studied population. The transmission of these parasites occurs by ingestion of faecally contaminated food and dog's faeces contaminated soil.

Of the total dogs studied 92.1 % were found infected with one or multiple parasitic infection in the present study. No significant difference was noted among all the breeds of dogs (hunter dogs, fighter dogs, German shepherds, puppy dogs as well as mixed breeds). However, the intensity of infection was high in-house hold dogs and they harbored more species of helminths than the other breeds. All the breeds of dogs were infected with protozoan parasites also, but the intensity of helminth infections found in domestic dogs was high. This could be due to the unawareness of the dog owners due the lack of access to the commonly used anti- helminthic drugs used.

Gastro-intestinal parasites of dogs are widely prevalent in the canine host of Swabi, Pakistan. It is found to be 92.1 % in prevalence in present study. The present study findings have also been matched to different studies conducted in varios parts of the world as: 82.5% (Mukaraterwa and Singh, 2010). 52.4% (Fontanarosa et al. 2006); 50% and above (Pok et al. 2000); 55% (Michel et al. 2015); 73.3% (Gugsa et al. 2015); 100%. Some of the study's results showed the high prevalence rate but low in respect with the present study findings as: 26.9% (Gracenea, 2009); 38% (Mustapha et al. 2016); 34.8%; 36.2% (Rojekittikhun et al.2014) were have been reported. The frequency of occurrence of these parasitic infections out of Pakistan is also low in prevalence. Other studies conducted showed the lowest rate of infection as: 6.7% (Gharkhani et al. 2014); 7.14% (Mirzaei and Fooladi, 2012) were also have been investigated.

Among the protozoan parasites found in our study included, *Coccidia spp*, *Isospora spp*, and Giardia *spp*, responsible for important zoonotic diseases. The result showed that dog fecal contamination with theses parasites pose an important zoonotic transmission to human and play a role in public health problem of the region.

Giardia is an intestinal protozoan parasite that is frequently detected in humans and animals. It resides in the small intestine of susceptible hosts, where it replicates. The morphological group of

importance for humans and domestic animals is *G. lamblia.*

Table 1: Pattern of poly-parasitism in different bread of dogs studied in district Swabi, Pakistan

Species of parasites	No. of dogs Infected (%)
With 2 species (7.03%, n=9)	
Isospora spp., Ascaris lumbricoides	3(2.34)
Taenia spp., Isospora spp.,	2(1.56)
Toxocara spp., Giardia spp.,	2(1.56)
Taenia spp., Giardia spp.,	2(1.56)
With 3 species (5.40%, n=7)	
Ascaris lumbricoid., Ancylostoma spp., Coccidian oocyst.,	1(0.78)
Coccidia oocyst., Taenia spp., Ancylostoma spp.,	1(0.78)
Toxocara spp., Coccidia oocyst., Taenia spp.,	1(0.78)
Giardia spp., Ascaris lumbricoid., Coccidia oocyst.,	1(0.78)
Coccidia oocyst., Ascaris lumbricoid., Toxocara spp.,	1(0.78)
Toxocara spp., *Ascaris lumbricoid., Isospora spp*.,	1(0.78)
With 4 species (5.40%, n=7)	
Diphyllidium spp., Coccidia spp., Echinococcus spp., Ascaris lumbricoid.,	1(0.78)
Coccidia spp., Trichuris spp., Toxocara spp., Ascaris lumbricoid.,	1(0.78)
Coccidia spp., Taenia spp., Diphyllidium spp., Giardia spp.,	1(0.78)
Diphyllidium spp., Ascaris lumbricoid., Taenia spp., Giardia spp.,	2(0.78)
Giardia spp., Ascaris lumbricoid., Toxocara spp., Taenia spp.,	1(0.78)
Coccidia spp., Taenia spp., Strongiloid strecoralis. Ascaris lumbricoid.	1(0.78)
With 5 species (2.30%, n=3)	
Toxocara spp., *Ascaris lumbricoides,* Toxascaris spp., *Ancylostoma spp*., *Coccidia oocyst*.	1(0.78)
Strongyloides stercoralis., *Toxocara spp*., *Isospora spp*., *Ascaris lumbricoides, Taenia spp.,*	1(0.78)
Diphylidium spp., *Coccidia oocyst., Taenia* spp., *Ascaris lumbricoides, Isospora spp*.,	1(0.78)
Total dogs infected	26 (20.3)
Total dogs studied	128

Table 2: Overall prevalence of gastrointestinal protozoan parasites in faeces of different breeds of dog studied in district Swabi, Pakistan

Parasite	Number of infection	%	P value (column factor)	P value (row factor)
Helminth parasites				
Taenia spp.,	12	25	0.01 (12.96)	0.01 (71.93)
Ascaris lumbricoides	15	31.2		
Toxocara spp.,	9	18.7		
Diphylidium	4	8.3		
Echinococcus spp.,	1	2.0		
Toxascaris spp.,	1	2.0		
Trichuris spp.,	1	2.0		
Ancylostoma spp.,	3	6.2		
Strongyloid stercoralis	2	4.1		
Sub-total	48			
Protozoan parasites				
Coccidia spp.,	11	42.3	0.0187 (83.99)	0.2017(0.2017)
Isospora spp.,	7	26.9		
Giardia spp.,	8	30.7		
Sub-total	26			

Table 3: Breed wise prevalence of Gastro-intestinal parasites found in faeces of different breed of dogs.

Dog Breed	No Examined	No Positive	Prevalence (%)	P value (95% CI)
Fighter	47	8	17.0	0.0119
Hunter	17	3	17.6	
German shepherd	21	4	19.0	
Puppy	21	5	23.8	
Mix breed	22	6	27.2	
Total	128	26	20.3	

Table 4: Sex and age wise prevalence of gastro-intestinal parasites found in feces of different breeds of dogs

Factors	No.examined	No.infected	Prevalence (%)	P value (95% CI)
Sex	128	26	20.3	0.3286
Male	100	18	18	
Female	28	8	28.5	
Age	128	26	20.3	0.3905
<1year	20	6	30	
>1year	108	20	18.5	

The role of animals in the transmission of giardiasis is debated for many years. Most of the human infections are expected to be man-to-man transmission. However, now that it is clear that dogs can carry the same genotypes of *G.lamblia* as humans, dogs are potentially infective to humans and therefore a zoonotic hazard. An infection with *G.lamblia* in dogs is rarely associated with clinical illness, and if so, most cases are associated with kennel situations. (Robertson et al. 2000). Infection with Giardia can

occur by the faecal-oral-transmission of cysts, through poor hygiene, or by ingestion of cysts in contaminated water or food. (Katagiri & Oliveira-Sequeira, 2008) examined faeces of both stray and domesticated dogs in Brazil and showed an association between *Ancylostoma spp*. and Giardia *spp*., and a higher prevalence of both in stray dogs. This combination of infection was the most common in dogs harbouring two or more parasitic species. Giardia arose the first protozoan in the current study, with the prevalence rate of 13(39.3%).Other contributor reports regarding the same parasites was as: 1.3% (Leelayoova et al.2009); 9% (Fontanarrosa et al., 2006); 3% (Gillespie and Bradbury, 2017); 7.2% (Puebla et al.2015); 3.5% (Villeneuve et al.2015); 16.9% (Katagiri1 and Oliveira-Sequeira, 2008); 6.1% (Gracenea et al. 2009); 2% (Elom et al.2015); 0.3% (Torres-Chable et al.2015); 1.3% (Sardarian et al. 2015); 2.8% (Rojekittikhun et al.,2014); 0.95% (Jamal Gharekhani , 2014); 42.26% (Mahmud et al. 2014); 1.3% (Kohansal et al.2017); 6.06%, 16.7% (Sotelo et al.2013). Giardiasis has a worldwide distribution and transmission from dog to human makes it worth investigating the prevalence on the study area.

On the basis of faecal samples analysis, the frequency of *Isospora spp*. (Coccidiosis) in dogs involved in the study were 36.3% (n=12). Variable rate of prevalence was noted as: The frequency of *Isospora spp* in the existent study was fairly high related to the results of who described the prevalence of coccidiosis 8.7% in dogs. Prevalence rate of *Coccidia* shown by other researcher outside the country was quite less as compared to the present study as; 1.5% (Rodrıguez-Vivas et al. 2011); 0.6% (Gracenea et al. 2009); 14.75% (Mahmud et al., 2014); 3.52% (Romero et al. 2015); 13.1% (Oudni-M'rad et al. 2017); 6.33 % (Younas et al. 2014).

The next most protozoan parasite arose was Isospora spp, the prevalence rate of which were reported 08(24.2%). Other compatible and non-compatible infection rate shown internationally was as: 3% (Fontanarrosa et al.2006); 7.8% (Perera et al.2013); 3% (Elom et al.2015); 1.3% (Mukaratirwaa and Singh, 2010); 2.7% (Sardarian et al. 2014); 9.53% (Mateus et al. 2014);

This might be attributed to asymmetrical and irregular use of anti-coccidal medications, breeds, topographical surroundings and responsiveness of the dog owners about the illness.

Single worm species infection was less prevalent in present study whereas several species infections were more ordinarily identified. The current findings are in accordance with to the results of others (Sowemimo and Asaolu, 2008. Present findings were contrast with the studies conducted by Ramirez-Barrios et al. 2004.

In present study male dogs were slightly more infected than female ones (P>0.05). Present findings were similar to the findings reported by Katagiri and Oliveira-Sequeira, 2007 the frequency of parasitism in male and female adults showed no difference (P > 0.05). A study conducted by Sowmimo and Asaolu, 2008 no significant difference was found in the prevalence of intestinal parasites in male and female dogs (P>0.05). According to Fontanarrosa *et al.,* 2006 no statistical sign can't difference was observed in the prevalence of parasites in male and female dogs (P>0.05). According to Pam *et al.,* 2015 the prevalence in relation to sex shows that the male had higher prevalence than the female 44.0% and 28% respectively. Male dogs recorded slightly more prevalent 55.1% than female 48.2%, though the difference was not statistically different (P>0.05) Amissah-Reynolds et al., 2016. Eighty-four (68.8%) of the males were positive for helminth and 112 (74.6%) of the female dogs were positive (Salihu et al. 2013). The frequency analysis showed non-significant difference in both of the gender in dogs, however it was non-significant (P>0.05) and in relation to age, the frequency of *T. canis* in young animals (4/14) was higher (P < 0.05) than in adult ones (18/240).

Multiple parasite species were determined more in the present study compared to the single parasitic infection. The results shown by other, (Vanparijs *et al.,* 1991; Ramirez-Barrios *et al.,* 2004) were contrast to this study. The double, triple and quadruple association of parasite among the current study were detected frequently with respect to mono, and pentruple association. Giardia, *Coccidia* species among Protozoan, while *Ascaris lumricoides*, *Taenia*, *Toxocara* and *Ascaris lumbricoides,* among helminth parasites observed in this study with a high prevalence; this may be due to the fact that these parasites are best adopted to the climatic factor of the study area..

CONCLUSION

This is the first report of GI parasites in dogs from district Swabi, Pakistan. Results call for the realization, determination of health hazards for the rural and sub-urban communities of this area. The present study results show that male dogs were highly infected than female ones, dogs above one year were found more infected than

below one year. Regarding breed of dogs, fighter dogs were found more infected than others. The highest multiple parasitic infection calls for the urgent management and control of dog parasite infection and transmission to the general community in the study area.

Based on the findings of the current study we hereby suggest that local people should be informed and educated about the zoonotic importance of the parasites which were high, especially multiple parasitic infection. Secondly, this study was proposed to find out that to how much the environment is being made contaminated by dog to human health, and how much potential risk factor pose by dog to Municipality. The dog owners should be aware about dog gastro-intestinal parasitic infection, their transmission and control measure. Health awareness trainings should be arranged for farmers, closer collaboration between researchers, practitioners and public health authorities.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Author, BA with all co-authors carried out the study design. BA performed the search and sign up the manuscript. SAM and SA perform statistical analysis of the manuscript. WK and SY give a hand in fecal samples examination, parasites identification through books and standard keys. SND, MA and RI critically evaluated the manuscript. All the author approved the manuscript for final publication

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