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Assessing risk factors associated with prevalence of canine gastrointestinal parasitic zoonoses in Andhra Pradesh, India

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ABSTRACT

Prevalence and risk factors associated with gastrointestinal parasites in dogs from central coastal districts of Andhra Pradesh, India was estimated by coprological examination. On examination of faecal samples of 1,907 dogs by saturated zinc sulphate floatation, sedimentation technique and modified Ziehl-Nielsen staining, an overall prevalence of 52.2% was observed. Single species infections were more common (49.7%) than mixed infections (2.5%). A total of 11 different species were detected. The most prevalent species was *Ancylostoma canis* followed by *Toxocara canis*, *Cystoisospora ohioensis*, *Dipylidium caninum*, *Toxascaris leonina*, *Capillaria aerophila*, *Diphyllobothrium latum*, *Cystoisospora canis*, *Entamoeba* spp., *Trichuris vulpis* and *Cryptosporidium* spp. The overall prevalence was high in stray dogs (65.9%). The prevalence of *A. caninum* (31.6%) was significant in pet dogs. Significant relationship between the season and prevalence of parasites was observed, the infection being more prevalent during rainy season. Binary logistic regression analysis identified age, season and the living condition of dogs and lack of zoonotic awareness of owners as risk factors for different parasitic infection.

Keywords: Dogs, Gastrointestinal parasites, India, Prevalence, Risk factors

Gastrointestinal (GI) parasites are among the most frequently encountered disease causative agents in dogs intervening welfare of the dogs. Mostly the dogs are infected without apparent evidence of the parasite's occurrence. However, canine GI parasitic infections constitute a great concern in man as infected dogs are important definitive or reservoir hosts for several zoonotic parasites (Robertson *et al*. 2000). Parasitic zoonoses like hydatidosis caused by *Echinococcus* species, visceral and ocular larva migrans by *Toxocara canis* and cutaneous larva migrans by *Ancylostoma* spp. are acknowledged to be endemic in India (Traub *et al*. 2005), particularly among stray and semi-domesticated dogs. Besides, parasites like *E. granulosus* and *Sarcocystis* spp. use food animals as intermediate hosts in which they cause great economic loss through organ condemnation during meat inspection.

Accordingly, there is a dire need for periodic surveillance of these parasites within a given region for successful formulation and implementation of effective control strategies both in animals and man. In India, there seems to be a paucity of data on the prevalence of canine GI parasites and is restricted to some category of dogs (Traub *et al*. 2014, Moudgil *et al*. 2016). While no information on epidemiology of canine GI parasites from Andhra Pradesh (AP) is available, the current study is aimed to assess the prevalence and risk factors associated with GI parasitic infections in dogs from AP, India.

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MATERIALS AND METHODS

Sampling: The study area encompasses four central coastal districts of AP where the climate is tropical and humid (Supplementary Table 1). In order to have a representative sample of dogs from all the Animal Husbandry Divisions of the districts, a random stratified sampling method was used to select the areas and a total of 1907 faecal samples of dogs were collected over a period of one year (September 2017–August 2018) from East Godavari (245), West Godavari (511), Krishna (738) and Guntur (413) to know the prevalence of canine GI parasites during different seasons, viz. summer (March–June), rainy (July–October) and winter (November–February). Faecal samples were collected from the dogs at kennels and those visiting the veterinary hospitals and hence approval of institutional ethics committee was not warranted. Dogs were divided into three groups based on living conditions namely stray (free ranging dogs), pet (owned dogs, used as companion animals) and kennel (owned dogs, living in groups, used for reproduction). Samples from stray dogs were collected with the help of Local Municipal Corporation personnel in which age was estimated based on dentition and body size. The age of the dogs included in the study varied from two weeks to 15 years and were categorised into young (≤ one year) and adult (≥ one year). Breed was classified as pure-breed and Mongrel, and gender as male and female. Data base for each pet and kennel dog was obtained by giving questionnaire regarding age, sex, breed, living condition of the dog, anthelmintic history, origin/owner's address and owner's awareness on zoonoses transmitted from dogs to identify risk factors associated with GI parasitic infection.

Faecal examination: Each sample was evaluated for the presence of parasites by floatation using zinc sulphate (specific gravity 1.18) and sedimentation technique. Samples positive for coccidian oocysts were sporulated in 2.5% potassium dichromate solution and were distinguished by sporulation time and micrometry. *Cryptosporidium* spp. oocysts were identified by modified Ziehl-Nielsen staining. Parasitic infections were identified based on description of Bowman (2014) under light microscopy.

Statistical analyis: The prevalence of parasitic infections was assessed and significance in differences in frequencies was estimated by chi-square test. Binary logistic regression models using SPSS Statistics base 17 (2008) was adopted to explore the role of risk factors, viz. living condition, age group, gender, breed, medium, season and zoonoses awareness of owners on the frequency and type of parasitic infections. The data was subjected to multicollinearity test to ascertain non-collinearity between the independent variables and heteroskedasticity Glejser test to examine the dependency of variation of the error from a regression on the independent variables (factor). The model was performed on the dataset by setting the Living condition: Kennel; Age: Young; Season: Rainy; Sex: Female; Medium: Rural; Zoonotic awareness: Yes as reference fulfilling the assumptions and conditions of the model.

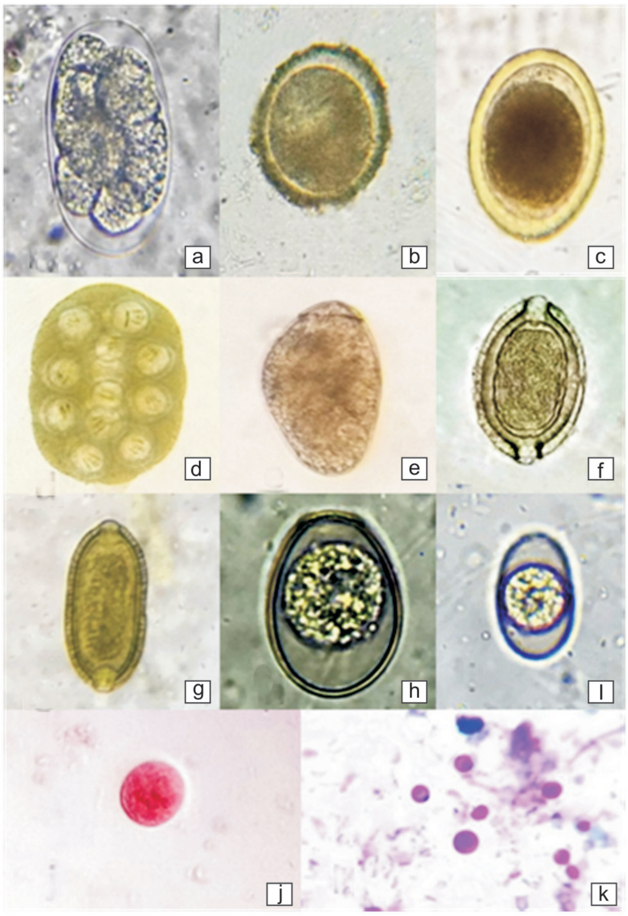

Fig. 1. Eggs of (a) *Ancylostoma caninum*, (b) *Toxocara canis,* (c) *Toxascaris leonine,* (d) *Dipylidium caninum*, (e) *Diphyllobothrium latum*, (f) *Tricuris vulpis*, (g) *Capillaria aerophila*, Unsporulated oocyst of (h) *Cystoisospora canis*, (i) *C. ohioensis*, (j) Cyst of *Entamoeba* spp., (k) Oocyst of *Cryptosporidium* spp.

RESULTS AND DISCUSSION

Out of 1907 dogs, 995 (52.2%) were parasitized with one or two of 11 different species of parasites (Fig. 1), each with higher rate in stray dogs (65.9%), that necessitates an effective anti-parasitic control programme.

Statistically there was no differences (P>0.05) between specific prevalence of each identified parasite and districts, thus indicating that these parasites were homogeneously distributed over the study area as there is no variation in climate (Supplementary Table 2). The high level of infection could be due to the lack of public awareness (93.4%) on zoonoses and strategic deworming programmes, both in rural and urban areas.

The overall infection with helminths was more frequent (P<0.001) than protozoan infections, with a ratio of 15:1, and was even higher in adult (21.4:1) dogs (Singh *et al*. 2011). While traditional coprological techniques are less sensitive it is anticipated that the true ratio between helminths and protozoa is lower as opined by Mircean *et al*. (2017). But the proportion of stray dogs harbouring intestinal protozoa (5.7%) was higher (P<0.01) than other category dogs. Contrary, an increasing tendency of protozoan infections than helminths were observed in developed countries, which could be through the practice of prophylactic anthelmintic therapy of owners who are aware of canine parasitic zoonoses (Schantz 1999).

Infection with single parasite (49.7%) was more commonly observed (P<0.001) than multiple species infection (2.5%). In mixed infections, exclusively double infections with two species were frequent (2.3%) and the most common mixed infection was the relation of different nematode species especially in young dogs, followed by association between nematode and *Entamoeba* spp., which differed significantly (P<0.001). The overall prevalence (P<0.001) and co-infections (P<0.01) were significant in young dogs (Supplementary Table 2). These statistics are of significance in veterinary practice to avoid abuse of drugs.

Prevalence of each parasite and associated risk factors are given in Tables 1 and 2.

Among the parasite identified, *A. caninum* was the most prevalent parasite, with a high prevalence in pet dogs, occurring in single or mixed infections. It has been reported as one of the most common canine parasites in India (Traub *et al*. 2014), especially in stray dogs. Further, larvae of *A. caninum* are involved in human cutaneous larva migrans that is endemic in India (Paul and Singh 2017). Currently, the risk factors for ancylostomosis were represented by age of dogs (≤ one year; P<0.001), living condition (pet and stray dogs; P<0.001), season (winter; P<0.001) and gender

Table 1. Per cent prevalence of individual parasite by living condition and season

Parasite	Total	Living condition			Season		
	(n=1907)	Kennel dogs (n=495)	Pet dogs (n=1113)	Stray dogs (n=299)	Winter (n=826)	Summer (n=571)	Rainy (n=510)
Helminths	51.2# (977)	31.9 (158)	57.4*** (639)	60.2*** (180)	51.6 (426)	40.8 (233)	62.4** (318)
A. caninum	27.4	18.6	31.6***	26.1***	31.2***	20.0	29.5
T. canis	19.1	11.7	21.0***	24.4***	15.0	18.0	27.1***
T. leonina	1.2	0.8	1.3	1.3	0.6	0.2	0.4
D. caninum	1.6	0.8	1.5	3.0	2.2*	0.4	2.0
D. latum	0.7	0	0.5	2.7###	1.1	0.2	0.8
T. vulpis	0.3	0	0.4	0.7	0.1	0	1.0
C. aerophila	0.9	0	1.0	2.0#	0.4	1.6	1.0
Protozoa	65 (3.4)	9 (1.8)	39* (3.5)	17* (5.7)	37*** (4.5)	9 (1.6)	19 (3.7)
C. canis	0.7	0	0.7	1.7#	1.2#	0.4	0.2
C. ohioensis	1.8	1.0	1.8	3.3	2.3	1.2	1.8
Entamoeba spp.	0.7	0.8	0.7	0.3	0.7	0	1.4#
Cryptosporidium spp.	0.2	0	0.3	0.3	0.2	0	0.4
Total		33.7	60.9***	65.9***	56.1	42.4	66.1***

Logistic regression model *P<0.05; **P<0.01; ***P<0.001. #Chi-square. Figures in parenthesis indicate frequency.

Table 2. Per cent prevalence of individual parasite by age, gender, breed and medium and identified risk factors

Parasite	Age		Gender		Breed		Medium	
	≤ 1 year (n=1071)	≥ 1 year (n=836)	Female (n=992)	Male (n=915)	Pure breed (n=1193)	Mongrels (n=714)	Urban (n=1185)	Rural (n=722)
Helminths	69.6*** (745)	27.8 (232)	50.3 (499)	52.2 (478)	52.1 (622)	49.7 (355)	51.7 (613)	50.4 (364)
A. caninum	36.1***	16.1	24.7	30.3***	27.2	27.7	27.6	27.0
T. canis	26.8***	9.3	20.8	17.4	20.2	17.4	19.4	8.7
T. leonina	1.8*	0.5	1.3	1.1	1.2	1.3	1.2	1.2
D. caninum	2.3**	0.6	1.7	1.4	1.5	1.7	1.5	1.7
D. latum	0.9	0.5	0.6	0.9	1.0	0.3	0.7	0.8
T. vulpis	0.5	0.1	0.3	0.3	0.4	0.1	0.4	0.1
C. aerophila	1.1	0.6	0.9	0.9	0.7	1.3	0.9	0.8
Protozoa	5.0*** (54)	1.3 (11)	3.3 (33)	3.5 (32)	3.6 (43)	3.1 (22)	3.3 (39)	3.6 (26)
C. canis	1.1*	0.1	0.4	1.0	0.7	0.7	0.5	1.0
C. ohioensis	2.6**	0.8	0.2	1.6	2.0	1.5	1.8	1.9
Entamoeba spp.	0.9	0.4	0.6	0.8	0.8	0.6	0.8	0.6
Cryptosporidium spp.	0.4	0	0.3	0.1	0.2	0.3	0.3	0.1

Logistic regression model. *P<0.05; **P<0.01; ***P<0.001. Figures in parenthesis indicate frequency.

(male; P<0.001). Prior studies labelled the age (Batchelor *et al*. 2008) and living condition (Mircean *et al*. 2017) as risk factors reporting higher values in young and shelter dogs. The high prevalence of ancylostomosis found in this study and those of earlier studies in India corroborating with human infection warrants implementation of control measures to address this condition.

Toxocara canis was the second most prevalent species, with a high prevalence in stray dogs and in puppies. Mostly, *T. canis* infections are common in dogs less than 6 months age and remains threat to children globally including in India (Jagannath *et al*. 2009) as is responsible for larval migrans. Higher prevalence of toxocarosis in stray population demarcates great zoonotic risk as public places are contaminated with the eggs that in turn remains source of infection to even pet and kennel dogs. Age was the risk factor identified for toxocarosis (≤ 1 year; P<0.001) in accordance to the observation of Overgaauw and Van Knapen (2013) in addition to season (Rainy; P<0.001) and living conditions (stray dogs, pet dogs; P<0.01) of dogs.

Capillaria aerophila, a trichurid parasite was detected in stray (highest 2.0%, P< 0.05) and pet dogs. Though, it is subclinical in canine and feline hosts, occasionally can infect humans (Aftandelians *et al*. 1977). As *C. aerophila* has been recorded usually in the last few years (Traversa *et al*. 2009, Mircean *et al*. 2017), it is suggested to reconsider its role in the veterinary and public health perspective.

The prevalence of *Toxascaris leonina* was lower in all dog categories (0.8–1.3%) compared to *T. canis* and was significantly high in young ones (P<0.05). The ratio between these two nematodes is ever higher in support of *T. canis*. Contrary, studies from Spain (Martinez-Moreno *et al*. 2007) reported higher prevalence of *T. leonina* compared to *T. canis*. As in the current study age (≤ one year OR=0.26) is reported to be a risk factor for toxascariosis (Barutzki and Schaper 2011).

The prevalence of *Trichuris vulpis* was low as also reported by Singh *et al*. (2011) and was significantly high in stray dogs than in pets, while the infection was nil in kennel dogs. However, Traub *et al*. (2014) reported absence of *T. vulpis* in stray and refuge dogs sampled from climactically distinct locations in India. No factor influenced (P>0.05) the prevalence of trichuriosis but earlier studies reported age as the risk factor (Batchelor *et al*. 2008).

The infection with *Dipylidium caninum*, a zoonotic tape worm was noticed in all category dogs (P>0.05). Various studies in India indicated prevalence of dipylidiosis in dogs (Moudgil *et al*. 2016) and in children who were in close association with dogs (Narasimham *et al*. 2013) emphasizing its zoonotic importance. The present study identified age (≤ one year OR=0.26) and season (winter; P<0.05) as the risk factors. The prevalence of dipylidiosis was higher (P<0.001) in stray dogs (Abere *et al*. 2013). Reports on diphyllobothriosis from dogs and human (Ramana *et al*. 2011) in India indicate that the ingestion of fresh or undercooked fish could be a risk.

Cystoisospora spp. was the most common protozoan found in dogs (2.5%), with *C. ohioensis* (1.8%) and *C. canis* (0.7%). The difference between the prevalence of *C. ohioensis* by dog category and season was not significant but, infection of *C. canis* was significantly (P<0.05) higher in stray dogs and winter. Young age represented the single risk factor for *C. canis* (OR=0.12; P<0.05) and *C. ohioensis* (OR=0.32; P<0.001) infection in accordance to Martinez-Moreno *et al*. (2007).

Except the season (rainy season; P<0.05), no factor influenced the prevalence of *Entamoeba* spp. There is no published report on canine amoebiasis from India though it was recorded in other parts of globe (Gillespie and Bradbury 2017) from pups.

The very low prevalence of *Cryptosporidium* sp. (0.2%) in the present study was in line with other studies (0.2 to 1.9%) (Paoletti *et al*. 2015, Utaaker *et al*. 2018). Though, *Cryptosporidium* dog genotypes were not detected in India, occasional reports of infection with zoonotic species such as *C. canis* in developing (Xiao *et al*. 2007) countries indicated the possibility of the transmission of cryptosporidiosis among human and dogs. However, molecular screening of *Cryptosporidium* spp. (Osman *et al*. 2015) in pets in France revealed the low prevalence of parasite suggesting a negligible role of dogs as zoonotic reservoirs. Though, there was no difference (P>0.05) in prevalence of cryptosporidiosis between age category oocysts were identified exclusively from young dogs.

The living condition of the dogs, age, season (Tables 1 and 2) and lack of zoonotic awareness of owners were identified as risk factors for different parasitic infection. Overall, the pet (P<0.001), stray (P<0.001) and young dogs (P<0.001) were 3.3, 3.4 and 6.7 (OR=0.15) times respectively, more prone to be infected than in reference groups (kennel and adult animals). The strategic deworming of kennel dogs with broad-spectrum anthelmintic combinations might be the reason for low prevalence of infection in these dogs compared to that of pet and stray dogs. The prevalence of all the parasite species showed a significant decreasing trend with age (Table 2). Gender, breed and medium did not influence the prevalence of infection with any parasite except for *A. caninum* which was more prevalent in male dogs (P<0.001) compared to female. The overall prevalence during rainy season (P<0.001) was 1.3 (OR=0.75) and 3.1 (OR=0.32) times respectively, more than that in winter and summer.

Similarly, regarding helminth parasites, the infection in pet, stray and young dogs (P<0.001) was 3.0, 3.2 and 5.5 (OR=0.18) times respectively, more infected than that in reference groups. The overall prevalence of helminths during rainy season (P<0.01) was 1.3 (OR=0.73) and 2.7 (OR=0.369) times respectively, more likely than in winter and summer. The protozoan infection in pet, stray (P<0.05) and young dogs (P<0.001) was 1.8, 3.1 and 3.8 (OR=0.26) times respectively, more than in reference groups. The overall prevalence of protozoa during winter (P<0.001) season was 1.4 times more compared to rainy.

The information provided by owners suggested that, 45.6% (508) of pet dogs and all kennel dogs have received anthelmintic treatment, and among these, kennel dogs have been treated with anthelmintics at every 4 months. Pet dogs received treatment once or twice in a year either with benzimidazoles or macrocycliclactones as a routine practice. Kennel dog owners and 457 responders from pet dog owners, answered the questionnaire intended to evaluate the level of awareness on canine parasitic zoonoses. Only 6.6% (30/457) pet owners and all kennel dogs owners had awareness on rabies and knew measures to avoid human contamination. Overall, questionnaire results reflected a general lack of knowledge towards canine parasitic zoonoses in accordance to the similar problems that have been reported in other investigations in India (Traub *et al*. 2005). The overall prevalence and prevalence of helminth parasites in pets of owner with lack of zoonotic awareness were 1.3 times more (P<0.05) than the pets of those owners who have awareness.

Overall, the prevalence of canine GI parasites that pose a zoonotic risk in public in Andhra Pradesh, India is significantly higher in pet dogs. The data generated on the prevalence and risk factors associated with these parasites contributes valuable evidence for the veterinarians to evolve accurate strategies for treatment and control of parasites and to reduce the habitat contamination with infective stages. Results revealed lack of zoonotic awareness in owners, and veterinarians should play a key role in increasing the level of public awareness on canine zoonotic parasites to alleviate public health risks.

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