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SUMMARY

The canine intestinal parasitosis has rarely been documented in Madagascar. Intestinal parasite causes one of the most digestive diseases in dog, and contributes to the risk of zoonoses due to the existing close relationship between humans and dogs. The main objective of the study was to determine the prevalence of canine intestinal parasitosis in Antananarivo and the associated risk factors for humans. This is a retro-prospective, analytical study covering the period between 2016-2017. Multivariate logistic regression was used to study the relationship between parasitic infestation and epidemio-clinical parameters. The prevalence obtained was 52.43% (n = 215/ 410). In general, the parasitic infestation was dominated by nematodes; the main identified parasites were *Toxocara canis* (73%) and *Ankylostoma caninum* (24.63%). The others parasites were *Trichuris vulpis* (2.78%), *Dipylidium caninum* (2.78%), *Toxascaris leonina* (0.93%), *Taenia spp* (0.46%). The results confirm the existence of canine gastrointestinal parasites and calls for the implementation of preventive measures.

Key words: Dog; Zoonosis; Intestinal parasitosis; *Toxocara canis*; *Ankylostoma caninum*

INTRODUCTION

The dog (*Canis familiaris*) is a domestic animal that maintains close contact with humans and other animals, so any lack of surveillance and vigilance on canine gastrointestinal parasites can promotes the transmission of zoonotic and non-zoonotic helminths (Bruzinskaite *et al*., 2009) Helminthiasis, can become a serious animal and public health problem (Baneth *et al*., 2016). Certain intestinal parasites are a potential source of infestation in humans and /or domestic animals such as hookworm, roundworm and protozoa by oro-faecal transmission cycle (Baneth *et al*., 2016; Deplazes *et al*., 2011; Karshima *et al*., 2020). In fact, dogs are effective sentinels in investigating the occurrence and epidemiological impact of zoonotic parasites (Lee *et al*., 2010). The type of parasite, and abundance depends on the environment, intermediate hosts availability, infectious capacity of the parasite and characteristics of the host (Dominique Grandjean *et al*., 2010; Johnson *et al*., 2015). The host factors include the complexity of the digestive tract, the amount of food consumed, and immune system response. Among the gastro-intestinal parasites affecting dogs, roundworm and hookworms causes a significant threat to animals and humans (Karshima *et al*., 2020).

To reduce the risk of transmission to humans, it has been proposed that, national program should be put in place by the veterinary service to harmonize and coordinate efforts that can lead to an effective fight against intestinal parasites (Overgaauw & van Knapen, 2013). This may include periodic fecal collection and examinations to identify intestinal parasites in dogs and establish an anthelmintic prevention and treatment regimen (Lee *et al*., 2010; Sager *et al*., 2006). To date, studies conducted in different continents have shown that, the prevalence of canine intestinal parasitosis varies according to the region studied, but the general prevalence ranges between 3.5% and 34% (Johnson *et al*., 2015). In Africa, the prevalence is twice as much compared to other continents (Abere *et al*., 2013). In Madagascar, rareness of scientific publications on canine gastrointestinal parasitosis constitutes a major obstacle to estimate the risk of these parasites in veterinary public health.

Having sufficient data based on scientific studies is of dual interest. First, will help to increase the understanding and consequently use the knowledge to improve the management of parasitized dogs and estimate the risk of transmission of zoonotic diseases (European Scientific Counsel Companion Animal Parasites, 2013; Overgaauw & van Knapen, 2013). The objective of this study was to determine the prevalence of intestinal parasitosis in domestic dogs, and to assess the risk factors for the transmission of these in others animals and humans

MATERIALS AND METHODS

The study was carried out in a VETCARE Madagascar a veterinary clinic, specializing, in the care of companion animals, located in the capital of Madagascar (Supporting Figure 1). The dogs examined were dogs of all ages and different canine breeds. The choice of the clinic was favored by acceptance of the veterinarian in charge of the clinic to carry out the study within the establishment, the provision of equipment for the diagnosis of intestinal parasites.

Retrospective and prospective study of intestinal parasitosis

The retrospective study of files of cases attended to identify cases of parasitized dogs from January 2016 to August 2017 was done. Records of dogs included in the study were those which showed that the dogs consulted were parasitized after coproscopic analysis. Records without or with unknown coproscopic result were excluded. The prospective study included dogs consulted, treated at the clinic, and having undergone a coproscopic analysis and treated during the month of September to December 2017. Animals brought at VETCARE clinic came from different areas within the urban and peri-urban areas of Antananarivo. Parasitized dogs, treated without a follow-up visit were not included in the study. In addition, the study excluded dogs treated with antihelminths for less than a week before the respective animals was brought at the clinic for consultation, and dogs with incomplete records. Examination of dogs for gastrointestinal parasites was conducted as previously described (Idika *et al*., 2017).

The stool was taken directly or indirectly. Direct sampling consisted to remove the stool from the dog's rectum by rectal search using a spatula. Indirect sampling consisted to take the stools emitted by the dog in the consultation room (Ngetich Wyckliff, 2017). A macroscopic examination was performed on all fecal samples to see adult form and proglottids of cestodes (Molina *et al*., 2003), then an optical microscopy examination was carried out after concentration of helminth eggs by sieving and the flotation methods for the qualitative analysis (Cringoli, 2004). The faecal samples were weighed using a precision balance to obtain 4 -5g of sample, and a portion of 1g was taken and placed in a disposable container, and diluted with saline solution (d = 1, 2). The mixture was sieved in a beaker and then transferred to a test tube, with a capacity of 20ml, up to the brim and covered with a coverslip and then let stand for 15 minutes.

The counting of eggs per gram (EPG) was done as previously described by Stoll (Stoll, N.R, 1923). Briefly, fecal sample was mixed with 42 ml of saline solution and homogenized. Homogenized sample was transferred into a test tube and left on the table for 05 minutes. Aproximately 0.15ml of the mixture was used for counting EPG. The EPG was counted according to the following formula: N = n x C x 100 where: N = number of eggs per gram of faeces, n = average number of eggs counted in 0.15ml of solution, C = multiplying coefficient according to the quality of the faecal sample emitted by the parasitized dogs (Dominique Grandjean *et al*., 2010; Stoll, N.R, 1923). The process was repeated twice, and the average was recorded.

Risk factors

The study variables were age, sex, race, location, lifestyle, vaccination, and deworming. The evaluated clinical parameters were: general condition, thermometry, state of dehydration, clinical signs, characteristics of parasitism (class of parasites, type of parasitism, season of infestation, parasitic elements and the number of eggs per gram of faeces (E.P.G), care characteristics, and the characteristics of dog's health after treatment (the course and the outcome of the disease).

Data analysis

Data analysis was performed with R studio® statistical software, version 3.4.2, 64 bits. Logistic regression with several explanatory variables (Generalized Linear Model or GLM, binomial family) was performed to study the relationship between the variable models and the parasite infestation. The variables selected in each model were tested to see the main effect and the interactive effect of certain variables studied on the presence of parasites. The probability threshold α is equal to 0.05: if $p \leq 0.05$ (significant relationship); $p > 0.05$ (no significant relationship).

Akaike Information Criterion with adjustment or AICc was used for the selection of the model to be studied (null model or model tested). It consists of a random analysis of the combinations of the explanatory variables studied.

RESULTS

Characteristics of the study population

During the study period, 215 out of 410 examined dogs were parasitized which is equivalent to, a prevalence of 52.43%. Mortality of 7.91% was recorded from the parasitized dogs. Males were the most parasitized at 55.35% of all the cases. Age wise, puppies were the most affected by helminths with a proportion of 44.20% with an average age of 3 months (1 month - 6 months) (Table 1). The study presented 52 dogs no specified age. Purebred dogs made up ¾ of the study population (74.90%), and parasitized dogs were located in urban areas for ¾ of the study population and had an outdoor lifestyle for nearly half of cases (Table 2). Deworming and vaccination were performed respectively in 30.23% and 33.95% of the 215 dogs infected, 190 dogs presented with illness or deterioration in health. The clinical signs detected during the examination are grouped into three categories, the proportion of each of which is 63.2% (n= 135) for digestive signs, 17.70% (n= 38) for respiratory signs and 37.61% (n = 81) is attributed to the other clinical signs not studied. Board. Abdominal pain is the best predictor of infestation in dog and his prevalence was 11,62% (n=25). Unspecified clinic sign was identified in 9 case (n= 9).

Table 1. Age average (in month) of parasitized dogs during the 2016-2017

	Min	1st quartile	Median	Average	3th quartile	Max
Dogs	0,96	2,53	4,66	21,00	11,06	182,63
Puppy	0,96	2,00	2,83	2,93	3,8	5,76
Junior	5,96	7,52	9,05	10,38	11,61	24,86
Adult dog	27,90	47,92	75,00	91,68	138,12	182,63

Table 2. Area and lifestyle of the parasite dog

		Number (n)	Proportion (%)
Breed	Purebred dog	161	74.90
	Common breed	37	17.20
	Crossed breed	17	07.90
	Total	215	100.00
Area	Urban	160	74.40
	Peripheral	48	22.30
	Mixed[1]	07	03.30
	Total	215	100.00

[1]: *group of dog which live in the urban area and move to the peripheral area where the owners hold a second house (the opposite case is also possible)*

Table 3. The proportion of cases involving single parasitism and parasite co-infections was examined for the years 2016 and 2017.

Species		Number	%
Single parasitism		144	66,98
	Ankylostoma caninum	18	08,37
	Toxocara canis	118	54,89
	Trichuris vulpis	02	00,93
	Toxascaris leonina	00	0,00
	Dipylidium caninum	04	01,86
	Taenia spp	00	00.00
	Protozoan (coccidia)	02	00,93
Unknown *(larval stage)*		31	14.42
co-infections		40	18.60
	Ankylostoma caninum + Toxocara canis	30	13,95
	Ankylostoma caninum + Dipylidium caninum	01	0,46
	Ankylostoma caninum + Toxocara canis+ Trichuris vulpis	03	1,39
	Ankylostoma caninum + Toxocara canis+ Taenia spp	01	0,46
	Toxocara canis + Dipylidium caninum	01	0,46
	Toxocara canis + Toxascaris leonina	02	0,93
	Toxocara canis + Trichuris vulpis	01	0,46
	Toxocara canis + Coccidia	01	0,46

Table 4. Mean of Counted Egg per gram or EPG from the feces 'sample of dog (n=35) during the prospective study

Species	Sample number	Mean of EPG	Standard deviation	Minimum	Maximum
Ankylostoma caninum	22	7210,53	14745,62	1000	51200
Toxocara canis	23	9123,68	19489,70	100	92000
Trichuris vulpis	3	57,89	202,193	600	800
Coccidia	3	1577,14	8647,308	200	51200

Table 5. Sociodemographic features associated with *Ankylostoma caninum* infestations in parasitized dogs observed in clinic in 2016 and 2017 (p-value < 0.05).

Factors (*n* = 215)	**Prevalence (%)** *Ankylostoma caninum*	Others	***p***[a]	**Odds Ratio**[a]
Lifestyle				
Outdoor*	40,00	60,00		-
Indoor	**97,26**	02,74	**$2{,}1.10^{-5}$**	0,03
Mixed	24,33	75,67	0,05	0,4
Age's category				
Puppies*	30,53	69,47	-	-
Junior	07,89	92,11	0,25	0,41
Adult	13,33	86,67	0,95	1,03
Non précisé	**32,70**	67,30	**0,03**	3,00
Sex				
Female*	18,18	81,82		
Male	**29,41**	70,59	**0, 03**	2,42
Unknown	25,00	75,00	0,87	0,83
Breed				
Common breed*	48,65	51.35		
Crossed breed	29,41	70,59	0,56	1,60
Purebred	18,63	81,37	0,05	0,40
Vaccination				
Vaccinated*	10,96	89,04		
Unvaccinated	**41,67**	58,33	**0,002**	5,93
Others	21,43	78,57	0,44	1,51

** : Reference factor, [a] : ajusted*

Characteristics of intestinal parasitosis

Toxocara canis was the frequently identified parasite (73%), followed by *Ankylostoma caninum* (24.63%), Trichurisvulpis (2.78%), *Dipylidium caninum* (2.78%), Coccidia (1.39%), *Toxascaris leonina* (0.93%) and *Taenia spp* (0.46%). The parasitic elements observed were categorized into two groups, as microscopic elements (eggs, larvae) and macroscopic elements (adult worms, ovigerous segments). 83.25% of intestinal parasites dog has been mainly identified from the microscopic elements of the stool (n = 179). Parasitized dogs did not shed macroscopic parasitic elements in 93.6 5% (n = 202) of cases. The larval forms represented 14.42% of cases. The coproscopic analysis showed at most an association of 03 parasitic species (Table 3). Quantitative coproscopy was performed on stool samples obtained during the prospective study (n = 35). The Table 4 shows the result of the quantitative analysis of parasitic infestations in dogs. Prior to the treatment of dogs, the number of Eggs per gram of faeces mean or Eggs/g ranged from 57.89 to 9123.53. A high level of infestation has been identified and demonstrated in during Toxocarosis (Eggs/g max = 92,000).

Analysis association between variables and parasite infestation

The association between the socio-demographic parameters and the parasitic species showed that the males were more likely to be infested by *Ankylostoma caninum* than the females (OR = 2.42; pa = 0.03). For medical prophylaxis, Table 5 shows that non-vaccination favorised the infestation by *Ankylostoma caninum* (OR = 5.93; pa = 0.002). Interior lifestyle was a protective factor against infestation by *Ankylostoma caninum* (OR = 0.03; pa = 2.1x10-05). With *Toxocara canis* infestation, the model retained was the null model (AICc = 242.16) after comparing with the AICc value of the model studied (AICc = 238.15). Statistical analysis showed a significant relationship between the null model and the infestation by *Toxocara canis* (p = 9.13x10-11).

The association between the clinical signs observed and the parasitic species identified demonstrated that the abdominal pain constituted a risk factor for infection by *Ankylostoma caninum* in dogs (OR = 2.5; pa = 0.04). Other intestinal parasites in frequent infestation with *Ankylostoma caninum* (pa = 6x10-5). With *Toxocara canis* infestation, the model retained was the null model (AICc = 242.16) after comparing the AICc value of the model studied (AICc = 250.67) with that of the null model. Statistical analysis showed that there is a significant relationship between the null model and the infection with *Toxocara canis* (p = 1.66 x 10-12).

DISCUSSION

The study revealed that dogs were exposed to intestinal parasites, and majority harbored parasites with zoonotic potential including *Toxocara canis* and *Ankylostoma caninum*. Thus, dogs infected with Toxocara. canis or hookworms represent a potential risk of environmental contamination and transmission of parasites to human (Davis, 1924; Le bars, 2014). The study also found male dogs to be the most parasitized than female dogs. This finding is comparable to previous studies conducted in 2014 and 2015 in the same area (Rakotonoely, 2015; Solofonandrianina Ravelojaona, 2014) and in Nigeria where parasites infestation in males was also higher although the difference was not significant (Idika *et al*., 2017). However, higher frequency of tissue migration in female adult dogs has been found to cause a false negative results of coproscopic analysis (Corda *et al*., 2019; Fahrion *et al*., 2008; Nijsse *et al*., 2016; Overgaauw & van Knapen, 2013), thus interpretation should be done with care.

Furthermore, among the parasitized dogs, majority were puppies (<06 months of age). This trend of younger dogs being more susceptible to parasitosis is not unique as it has been reported elsewhere (Beugnet & Guillot, 2000) and Alvarado-Esquivel *et al*., (2015). The parasitized dogs were mainly located in urban areas than peri-urban areas. This is different from the study in Gabon where intestinal parasitosis in rural areas was found to be higher (Beugnet & Guillot, 2000). The difference between urban and non-urban areas could be partly explained by differences in confinement levels. For example, unconfined dogs were as high as 48.84%, and 17% others with undefined confinement status. Indeed, earlier study in the same area revealed that, the number of parasitized dogs living indoors was lower than unconfined (Ravelojaona, 2014). In addition, statistical analysis showed that indoor lifestyle was a more protective factor against infection with *Ankylostoma caninum* (OR <1, (pa = 2,12x10-9) after controlling for other model factors (age, sex, breed and deworming status).

On the other hand, age was found to be a significant risk factor for *Ankylostoma caninum* infestation (OR = 3) although lack of information for non-owned dogs was one of the limitation. Other factors that have been reported to influence canine parasitosis include vaccination status and deworming, with the lack of vaccination being considered as a potential risk factor for parasite infestation (Alvarado-Esquivel *et al*., 2015).

Interestingly, 33.95% of dogs with parasitosis were those received anthelmintic prophylaxis. Unexpectedly, the study in Mexico also found that all the parasitized dogs were dewormed (Alvarado-Esquivel *et* *al*., 2015). The reasons for these unusual observations is not known, but could be partly related to practices of dog owners to self-medicate their dogs with questionable treatment accuracy. According to the type of parasites, 98.60% were helminths and the remaining proportion being cases for protozoa. This is contrary to observation's in Chile where protozoa were dominant compared to helminths (Lopez *et al*., 2006). The differences could be related to differences in animal factors and others which remains a subject of investigation. In most cases, dogs were found to be infested by a single parasite than polyparasitism as opposed to other study elsewhere which reported dogs infected with multiple parasites although *Toxocara canis* and *Trichuris vulpis* were the most prevalent (Alvarado-Esquivel *et al*., 2015). Quantitatively, on average, the EPG count was relatively higher but significantly lower than the amount reported elsewhere (Overgaauw & van Knapen, 2013).

Unlike in Mexico where the parasitized dogs were dominated by the crossbreeds 98.4% (n = 63/64), in this study, breed could not be determined in 87.80% of cases. Nevertheless, pure breeds are usually more valuable and are more likely to receive more care than others. Despite of the findings being instrumental in informing preventive and other measures against intestinal parasites of dogs, this study has some limitations. The fact that the study was only carried out within a single clinic in the capital, the results cannot be generalized for the entire canine population of the city and the country where the study was conducted, and the absence of certain data in the electronic registers could potentially lead to bias in certain results, and therefore the findings should be interpreted with care.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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