

# Prevalence of Major Digestive and Respiratory Helminths in Dogs and Cats in France: Results of a Multicentric Study.

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## Research Article

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# Abstract

**Background:** The real local distribution of helminths in dogs and cats and the evaluation of risk of contamination may represent an important challenge for veterinarians due to their effects on animal health and their potential zoonotic risk. The overall goal of this study was to estimate the current apparent prevalence of the digestive and respiratory helminths infecting in client-owned dogs and cats in France.

**Methods:** Faecal samples were collected from 414 dogs and 425 household dogs and cats, respectively, in 2017 - 2018 in 20 study sites and analysed by coproscopy. The sampling included specimens of both genders, various breeds, ages and varieties of living environments, lifestyles, and feeding regimes. Associations between parasitic infection and qualitative factors were explored.

**Results:** Overall, 125 (14.9%) samples (15.2% in dogs and 14.6% in cats) were positive for at least one of the nine species of helminths identified. Infection rates were the highest for *Toxocara canis* and *T. cati* (8.5% and 11.3%, respectively), while *Toxascaris leonina* was found only in one cat (0.2%). The prevalence of *Ancylostoma caninum* and *U. stenocephala* in dogs were 1.7% and 4.3%, respectively. No hookworms were found in cats. Whipworms (*Trichuris vulpis*) were identified in 2.7% of the dogs. Tapeworms (*Dipylidium caninum* and Taeniidae) were rarely found (<1% in dogs and <3% in cats). The prevalence of lungworms (*Angiostrongylus vasorum* in dogs and *Aelurostrongylus abstrusus* in cats) was below 1%. Significantly higher infection rates were identified in young individuals and in animals with outdoor access, living in countryside, and in intact animals (especially in cats). In addition, cats not fed exclusively with commercial diets and living with other animals (dogs and/or cats) were at higher risk of parasites. For dogs, hunting/herding and walking off-leash are additional risk factors. Furthermore, pets never dewormed had significantly more frequently parasites than pets dewormed 1-2 times per year.

**Conclusions:** The overall prevalence of helminths (some of which being zoonotic), the risk factors and the current low deworming frequencies reported in this study (20.5% animals never dewormed), 26.4% dewormed  $\geq 3$  times/year) illustrate the need for improving pet owners' adherence to anthelmintic guidelines in France.

## Introduction

Internal parasites of pets are of significant importance, not only because of their adverse effects on the health of dogs and cats but also due to their zoonotic potential. Helminth infections in dogs and cats can lead to a wide variety of clinical conditions including gastrointestinal or respiratory signs, coagulopathies, neurological disorders, anaemia, dermatitis, thinning and decreased body condition or performance. Severe cases can be fatal, especially in young or immunocompromised animals [1, 2]. Furthermore, *Toxocara canis* and *T. cati* are major parasites in domestic animals due to their fairly high prevalence, their adverse effects on young animals and because they may cause visceral and ocular *larva migrans* as well as neurological signs or potential booster effect on atopy in humans [1, 3, 4]. Other commonly

mentioned zoonotic helminths from carnivores include e.g., *Ancylostoma caninum*, which may be responsible for human cases of cutaneous *larva migrans*, the cestodes *Echinococcus* spp. involved in the cystic and alveolar echinococcosis, *Dipylidium caninum* and *Dirofilaria* sp. (*D. repens* and *D. immitis*)[5].

Because of the pathogenicity and zoonotic risks of the canine and feline helminths, several epidemiological studies have been conducted worldwide over the past thirty years. About twenty of those were conducted in France. Half of these French surveys covered a relatively small population of dogs and/or cats (<500 animals) and only focused on one type of parasite. Only three were prospective and multicentric, involving at least two different French regions [6–8] and only one study (published in 1997) included both dogs and cats. Updated data were desired.

The objectives of this study were to estimate the prevalence and diversity of digestive (roundworms, hookworms, whipworms, tapeworms) and cardio-respiratory helminths using fecal samples of dogs and cats in France and to identify the main risk factors associated with their presence in companion animals.

## Methods

### Study area and study population

This multicentric survey was conducted in France in 20 study sites, including the four National Veterinary Schools and 16 veterinary clinics distributed in 18 French departments spread across the country between November 2017 and July 2018. Each study site enrolled client-owned pets living within the corresponding geographic area. To be eligible for inclusion, the animals should not have left their department within the last 6 months, should not have been dewormed within the past 30 days, nor shown disorders requiring the use of a dewormer on the day of the visit.

### Faecal sample collection and parasitological procedure

At least 5 grams of faeces per animal were collected by the investigators (one designed practitioner per involved clinic, or per National Veterinary School) or by the owners immediately after defecation. Samples were then sent within 48 hours at ambient temperature to the laboratory of parasitology of the attributed National Veterinary School for analyses or were stored for a maximum of 72 hours at +4°C before shipment. The delay between sample collection and processing did not exceed 5 days. When appropriate, tapeworm segments visible to the naked eye were collected either by the investigator or by the owners and sent together with faecal samples at room temperature.

Faecal samples were inspected macroscopically and microscopically. A flotation method (modified from Raynaud 1970 [9]) with faeces diluted at 1:15 in saturated NaCl solution (specific gravity: 1.18) was used to assay the first aliquot (2 grams of faeces). A McMaster slide was filled and, after a few minutes, fully observed under a microscope. A second slide was also prepared by placing a glass coverslip on a tube filled with the remaining filtered solution for 30 minutes and then observed under a microscope (Obj. x10 - 40). The total number of eggs per parasite counted in the McMaster slide was multiplied by 15 to get the

corresponding number of eggs per gram (epg). A count of 7 epg was attributed when eggs were detected only on the second slide. The remaining faeces (maximum 25 g) were used to detect lungworm larvae using the McKenna sedimentation technique [10]. After 12 to 24 hours, the larvae were counted and identified on a slide under a microscope (x100-400). Tapeworm segments, eggs and larvae were identified under a microscope using usual morphological diagnosis criteria [11, 12].

## Statistical Analysis

The primary variable was defined as the presence or the absence of at least one parasite in each animal. In a first step, the Chi-squared test, or the alternative Fischer's exact test for low sample size, were used to detect possible associations of parasitic infection with different risk factors. We first tested for differences among the parasitology laboratories in the four National Veterinary Schools which may result from non-uniform parasite distribution in France or differences to detect parasites. Then, we tested an influence of animals' age (3 modalities:  $\leq 6$  months, 6 – 24 months,  $> 24$  months of age), sex (2 modalities: male, female), number of other pets in the house (2 variables: number of conspecific pets in the house, total number of dogs and cats in the house; each variable has 3 modalities: no other pet, 1-2 other pets,  $\geq 3$  other pets), food type (3 modalities: kibbles / wet only, Kibbles / wet with extra, homemade including raw meat), and yearly frequency of deworming (3 modalities: never, 1 – 2 times/year,  $\geq 3$  times/year) on the prevalence of parasites. We also tested for an influence of the reproductive status (2 modalities: intact, neutered/spayed), but only for individuals  $> 6$  months old, to avoid confounding effects of age and reproductive status, and as we expected the sex to influence parasite abundance mostly in sexually mature individuals.

Additional variables were considered. For cats, we tested for the influence of both the lifestyle and the living environment using a 3 modalities variable (indoor, outdoor access in the city, outdoor access in the countryside). For dogs, we tested for an influence of the living environment (city / countryside), the lifestyle (3 classes: indoors, outdoors with limited or unlimited contacts with other pets), the activity of dogs (pet, hunting/herding), the time spent outside without a leash (4 modalities: 0%,  $< 50\%$ ,  $50\% \leq x < 100\%$ , 100%), and observed coprophagy (yes / no). We also tested for a linear and quadratic influence of animal's age on parasite prevalence using logistic regressions and likelihood ratio tests (chi-squared tests).

**In a second step, using multivariate logistic analyses, all the risk factors with a significant influence ( $p < 0.05$ ), based on chi-squared test (or fisher test), were considered simultaneously to expand our understanding of the relationships among them considering all two-way interactions. Then, when an influence of the reproductive status was detected with Chi-squared tests, we tested for the influence of the reproductive status using the previously selected multivariate model on the data subset including  $> 6$  months old individuals. Model selections for multivariate logistic analyses were performed by comparing all the models including the different combination of variables using the Akaike's Information Criterion corrected for small sample size (AICc). We retained the model with the lowest AICc value, and when two or more competing models had a  $\Delta AICc < 2$ , we retained the simplest model according to the parsimony rule [13]. All analyses and model plots were performed using R 4.0 [14].**

# Results

## Dogs

A total of 414 faecal samples were analysed from client-owned dogs. The median age of dogs included in the study was 2.35 years [0.2; 14.2]<sub>95%CI</sub>, with 79.5% of the dogs older than 6 months of age (Table 1). The majority of the dogs included in the study were pet dogs (92.5%) and pure breed dogs (74%, n=305/412). Samples were evenly distributed between genders, with 51.4% of samples originating from male dogs, with 35.9% (n=60/167) and 53.1% (n=86/162) of male and female dogs older than 6 months of age being spayed/neutered, respectively. More than half of the dogs (55.1%) lived in a closed environment where the contact with other dogs or cats was limited (indoor or outdoor limited). The majority of dogs lived in the countryside (63.2%) and 40.4% of the dogs spent at least half of their outdoor time off-leash. Dogs frequently lived with at least another animal (dog or cat) in the same household (57.9%). Coprophagy was reported by 19.9% of dog owners. Most of the dogs (67.15%) were fed exclusively with kibbles and/or wet food and only 3.9% were fed with raw meat or homemade food. The percentage of dog owners never deworming their dog was 12.4%, including 32.5%, 8.9%, and 6.4% of  $\leq 6$  months, 6 – 24 months, > 24 months of age dogs, respectively.

Table 1

Sample sizes and apparent prevalence for each variable and modalities for dogs and cats, with results of univariate statistics on apparent prevalence of all helminth's species (NA = Not Available data)

Variables	Dogs				Cats			
	tot	App. Prev.	[IC]	p-value	tot	App. Prev.	[IC]	p-value
Age								
≤6 months	85	29.4%	[20.1-40.3]	***	58	34.5%	[22.5-48.1]	***
6-24 months	110	16.4%	[10.0-24.6]		163	18.4%	[12.8-25.2]	
>24 months	219	9.1%	[5.7-13.8]		204	5.9%	[3.1-10.0]	
Sex								
Male	213	12.7%	[8.5-17.9]	NS	196	13.8%	[9.3-19.4]	NS
Female	201	17.9%	[12.9-23.9]		229	15.3%	[10.9-20.6]	
Reproductive status if ≥ 6 months								
Intact	183	14.8%	[10.0-20.7]	·	104	22.1%	[14.6-31.3]	***
Neutered/spayed	146	7.5%	[3.8-13.1]		263	7.2%	[4.4-11.1]	
Other pets in the house								
Number of conspecific								
No	262	13.0%	[9.2-17.7]	NS	228	11.8%	[7.9-16.8]	NS
1 or 2	128	18.0%	[11.7-25.7]		147	17.0%	[11.3-24.1]	
3 or more	24	25.0%	[9.8-46.7]		47	19.1%	[9.1-33.3]	
NA					3			
Number of other dogs and cats								
No	174	12.6%	[8.1-18.5]	·	174	13.2%	[8.6-19.2]	*

*p-values*: ·:  $P < 0.1$ , \*:  $P < 0.05$ , \*\*:  $P < 0.01$  and \*\*\*:  $P < 0.001$ .

Variables	Dogs				Cats			
1 or 2	169	13.6%	[8.8-19.7]		178	11.8%	[7.5-17.5]	
3 or more	70	24.3%	[14.8-36.0]		70	24.3%	[14.8-36.0]	
NA	1				3			
Food type								
Kibbles / wet only	278	15.5%	[11.4-20.3]	NS	347	12.4%	[9.1-16.3]	*
Kibbles / wet + extra	120	15.0%	[9.1-22.7]		61	21.3%	[11.9-33.7]	
Other	16	12.5%	[1.6-38.3]		16	31.3%	[11.0-58.7]	
NA					1			
Lifestyle/living environment								
Indoor					202	6.9%	[3.8-11.4]	***
Outdoor in city					63	15.9%	[7.9-27.3]	
Outdoor in countryside					160	23.8%	[17.4-31.1]	
Living environment								
City	152	6.6%	[3.2-11.8]	***				
Countryside	261	20.3%	[15.6-25.7]					
NA	1							
Lifestyle								
Indoor	17	5.9%	[0.1-28.7]	NS				
Outdoor limited	211	15.6%	[11.0-21.3]					
Outdoor unlimited	186	15.6%	[10.7-21.6]					

*p-values:* : P < 0.1, \*: P < 0.05, \*\*: P < 0.01 and \*\*\*: P < 0.001.

Variables	Dogs				Cats			
Dog's activity								
Pet	383	13.6%	[10.3-17.4]	**				
Hunting/herding	31	35.5%	[19.2-54.6]					
Time walking off-leash for dogs								
0%	72	6.9%	[2.3-15.5]	*				
<50%	174	16.7%	[11.5-23.1]					
[50-100%[	131	14.5%	[9.0-21.7]					
100%	36	27.8%	[14.2-45.2]					
NA	1							
Coprophagy								
Yes	82	15.9%	[8.7-25.6]	NS				
No	330	14.8%	[11.2-19.2]					
NA	2							
Yearly frequency of deworming								
Never	50	32.0%	[19.5-46.7]	***	122	23.8%	[16.5-32.3]	**
1-2 times/year	173	10.4%	[6.3-15.9]		188	10.1%	[6.2-15.3]	
≥3 times/year	61	31.1%	[19.9-44.3]		130	24.6%	[17.5-32.9]	
NA	11				8			
<i>p-values:</i> ∴ P < 0.1, *: P < 0.05, **: P < 0.01 and ***: P < 0.001.								

From the 414 samples tested in dogs, 63 (15.2%) were positive for at least one helminth, including 62 (15.0%) with nematodes and only 2 (0.5%) with *Dipylidium caninum* (Table 2). Among them, 12 dogs (2.9%) had a mixed infection with 2 or 3 different helminth species. The most frequent helminths were *Toxocara canis* (8.5%; median: 84 epg, max: 12400 epg) and *Uncinaria stenocephala* (4.3%; median: 74

egg, max: 1850 egg; Additional file 1. Table S1). *Trichuris vulpis* and *Ancylostoma caninum* were present in 2.7% (median: 15 egg, max: 800 egg) and 1.7% (median: 100 egg, max: 500 egg) of faecal samples respectively, whereas *Angiostrongylus vasorum* and cestodes were each identified in 0.5% of the samples (Table 2).

Table 2  
Samples and apparent prevalence for each helminth's species in dogs and cats.

	Dogs			Cats		
	N	App. Prev.	[CI]	N	App. Prev.	[CI]
N total	414			425		
All nematodes (eggs and larvae)	62	15.0%	[11.7-18.8]	53	12.5%	[9.5-16.0]
Gastro-intestinal nematodes (eggs)						
<i>Toxocara canis</i>	35	8.5%	[5.9-11.6]	–	–	–
<i>Toxocara cati</i>	–	–	–	48	11.3%	[8.4-14.7]
<i>Toxascaris leonina</i>	0	0.0%	[0.0-0.9]	1	0.2%	[0.0-1.3]
<i>Ancylostoma caninum</i>	7	1.7%	[0.7-3.5]	–	–	–
<i>Ancylostoma tubaeforme</i>	–	–	–	0	0.0%	[0.0-0.9]
<i>Uncinaria stenocephala</i>	18	4.3%	[2.6-6.8]	0	0.0%	[0.0-0.9]
<i>Trichuris vulpis</i>	11	2.7%	[1.3-4.7]	–	–	–
Cardio-respiratory nematodes (larvae)						
<i>Angiostrongylus vasorum</i>	2	0.5%	[0.1-1.7]	–	–	–
<i>Aelurostrongylus abstrusus</i>	–	–	–	3	0.7%	[0.1-2.1]
Gastro-intestinal cestodes (eggs and segments)						
<i>Dipylidium caninum</i>	2	0.5%	[0.1-1.7]	8	1.9%	[0.8-3.7]
Taeniidae	0	0.0%	[0.0-0.9]	5	1.2%	[0.4-2.7]
Co-infestations						
Two or more parasites detected	12	2.9%	[1.5-5.0]	5	1.2%	[0.4-2.7]
Both nematodes and cestodes	1	0.2%	[0.0-1.3]	4	0.9%	[0.3-2.4]
<i>All helminth's parasites</i>	63	15.2%	[11.9-19.0]	62	14.6%	[11.4-18.3]

Univariate tests demonstrated significant correlations (all  $P < 0.05$ ) between the prevalence of parasites in dogs and the age (as a categorical variable; Fig. 1), the living environment, the proportion of time spent outside off-leash (Fig. 2), the activity of the dog (hunting/herding vs pet dog), and the frequency of deworming. Considering the age as a continuous variable, we had a significant linear decrease of the prevalence as dog aged (LRT [compared to the null model]:  $\text{Chi}^2 = 9.09$ ,  $\text{df} = 1$ ,  $P = 0.003$ ), and a quadratic decrease (i.e., a faster decrease of prevalence as dog aged in young dogs compared to adult dogs) close to significance (LRT [compared to the linear model]:  $\text{Chi}^2 = 3.55$ ,  $\text{df} = 1$ ,  $P = 0.056$ ). Considering only dogs  $> 6$  months old ( $n = 329$ ), the prevalence of parasites tended to be higher in intact compared to spayed/neutered dogs (14.8% vs 7.5%,  $P = 0.063$ ; Table 1, Fig. 1).

All the significant variables were tested in multivariate logistic analysis, including all two-way interactions. Based on data from 401 dogs, the selected model included the additive effects of the age, the activity of dogs, the living environment, and the proportion of time spent outside off-leash (Table 3). The prevalence of parasites decreased as dogs aged (Fig. 1), with 6 – 24 months old dogs having a lower prevalence than  $< 6$  months old dogs (OR = 0.38 [0.18, 0.81]<sub>95% CI</sub>) and a higher prevalence than  $> 24$  months old (OR = 2.0 [0.93; 4.26]<sub>95% CI</sub>; Tables 1 & 4). It was higher in hunting/herding dogs compared to pet dogs (OR = 2.81 [1.14; 6.75]<sub>95% CI</sub>), in dogs living in countryside compared to dogs living in city (OR = 3.37 [1.63; 7.61]<sub>95% CI</sub>), and in dogs being all the time off-leash compared to dogs never off-leash (OR = 5.11 [1.50; 19.34]<sub>95% CI</sub>; Fig. 2).

Table 3

Model selection of mixed-effects models based on corrected Akaike's Information Criterion (AICc) for testing the effects of different risk factors on parasitic infection in dogs and cats. *df* are the degree of freedom, *weight* are the Akaike weights. Only the ten first ranked models are presented. The final selected model is shown in bold.

Response variable	Model	df	AICc	$\Delta$ AICc	weight
<b>Dogs</b>	<b>Age + activity + LE + Off-leash</b>	<b>8</b>	<b>307.1</b>	<b>0.00</b>	<b>0.060</b>
	Age + activity + LE + Off-leash + LE*activity	9	307.3	0.21	0.054
	Age + YFD + activity + LE + Off-leash + age*activity + YFD*activity + LE*activity	15	308.2	1.18	0.034
	Age + activity + LE + Off-leash + age*activity + LE*activity	11	308.6	1.52	0.028
	Age + activity + LE + Off-leash + age*activity	10	308.6	1.57	0.028
	Age + YFD + activity + LE + Off-leash	10	308.7	1.65	0.026
	Age + YFD + activity + LE + Off-leash + age*activity + YFD*activity + LE*activity + Off-leash*activity	18	309.0	1.96	0.023
	Age + YFD + activity + LE + Off-leash + LE*activity	11	309.3	2.20	0.020
	Age + activity + LE + Off-leash + LE*activity + Off-leash*activity	12	309.3	2.22	0.020
	Age + activity + LE + Off-leash + Off-leash*LE	11	309.4	2.35	0.019
<b>Cats</b>	<b>Age + LLE</b>	<b>5</b>	<b>289.9</b>	<b>0.00</b>	<b>0.207</b>
	Age + LLE + age*LLE	9	290.9	0.99	0.126
	Age + food + LLE + age*LLE	11	291.9	1.94	0.078
	Age + food + LLE	7	291.9	2.00	0.076
	Age + YFD + LLE	7	292.1	2.12	0.071
	Age + NODC + LLE	7	293.2	3.21	0.041
	Age + YFD + LLE + age*LLE	11	293.4	3.49	0.036
	Age + NODC + food + LLE + NODC*food	13	293.7	3.74	0.032
	Age + NODC + LLE + age*LLE	11	294.0	4.07	0.027
	Age + NODC + food + LLE + age*LLE + NODC*food	17	294.2	4.23	0.025
NODC: number of other dogs and cats; LE: living environment; LLE: lifestyle/living environment; YFD: yearly frequency of deworming					

Table 4

Model estimates ( $\beta \pm$  Standard Error) of the selected models testing the influence of risk factors on parasitic infection in dogs and cats. Reference levels for each categorical variable were 6 – 24 months for Age, *pet* for Activity, 0% for the time spent outside without a leash (Off-leash), and, for dogs, *City* for Living Environment (LE), and, for cats, *indoor* for lifestyle/living environment (LLE).

Response variable	Variable	$\beta \pm$ SE	t-value	<i>p</i>
<b>Dogs</b>	Intercept	-3.32 $\pm$ 0.63	-5.29	***
	Age( $\leq$ 6 months)	0.96 $\pm$ 0.39	2.48	*
	Age(> 24 months of age)	-0.69 $\pm$ 0.38	-1.80	.
	Activity(Hunting/herding)	1.03 $\pm$ 0.45	2.29	*
	LE(Countryside)	1.22 $\pm$ 0.39	3.12	**
	Off-leash(<50%)	0.80 $\pm$ 0.53	1.50	
	Off-leash(50% $\leq$ x<100%)	0.28 $\pm$ 0.56	0.50	
	Off-leash(100%)	1.63 $\pm$ 0.64	2.54	*
<b>Cats</b>	Intercept	-2.54 $\pm$ 0.34	-7.47	***
	Age( $\leq$ 6 months)	1.03 $\pm$ 0.38	2.74	**
	Age(> 24 months of age)	-1.43 $\pm$ 0.38	-3.74	***
	LLE(outdoor access in the city)	1.36 $\pm$ 0.48	2.84	**
	LLE(outdoor access in the countryside)	1.68 $\pm$ 0.37	4.57	***
<i>p-values</i> : *: $P < 0.05$ , **: $P < 0.01$ and ***: $P < 0.001$ .				

## Cats

A total of 425 faecal samples were analysed from client-owned cats. The median age of cats included in the study was 1.8 years [0.2; 14.9]<sub>95%CI</sub> (range: 30 days to 19.0 years), with 86.3% of cats older than 6 months of age (Table 1). The majority of cats included in the study were European breed (87.6%). More than 46% of the enrolled cats were males, with 75.15% (n=124/165) and 68.8% (n=139/202) of male and female cats older than 6 months of age being spayed/neutered, respectively. More than 52% of the cats had free access to the outside. The majority of the animals lived in the countryside (57.2% of the cats). Most of the cats (58.8%) lived with other companion animal (cat or dog). The majority of the cats (81.8%) were fed exclusively with kibbles and/or wet food. The percentage of cats never dewormed before inclusion in the survey was 29.3%, including 71.9%, 27.0% and 18.9% of  $\leq$  6 months, 6 – 24 months, > 24 months of age cats, respectively. Most of the cats > 6 months of age were treated 1 – 2 times/year (39.0% and 60.7% for 6 – 24 months and > 24 months of age cats, respectively).

Parasites were detected in 62 (14.6%) faecal samples, including 53 (12.5%) by nematodes and 13 (3.1%) by cestodes (Table 2). Five cats (1.2%) had a mixed infection with 2 or 3 different helminth species, with 4 cats (0.9%) infected by both nematodes and cestodes. *Toxocara cati* was the most common helminth found in cats (11.3%; mean: 450 epg, range: 7 - 6000 epg; Additional file 1. Table S1) whereas *Dipylidium caninum*, Taeniidae, *Aelurostrongylus abstrusus*, and *Toxascaris leonina* were detected in less than 2% of faecal samples (1.9%, 1.2%, 0.7% and 0.2%, respectively).

Using univariate tests, we found significant correlations (all  $P < 0.05$ ) between the prevalence of parasites and the age (as a categorical variable; Fig. 1), the presence of other animals (cat and/or dog) at home, the living environment/lifestyle, the food type, and the frequency of deworming. Logistic regression showed a significant linear decrease of parasite prevalence with the age of the cats (LRT [compared to the null model]:  $\text{Chi}^2 = 32.27$ ,  $\text{df} = 1$ ,  $P < 0.0001$ ), but no quadratic relationship (LRT [compared to the linear model]:  $\text{Chi}^2 = 0.21$ ,  $\text{df} = 1$ ,  $P = 0.647$ ). Considering only cats  $> 6$  months old ( $n = 367$ ), we observed a significantly lower prevalence of parasites in spayed/neutered cats compared to intact cats (Fig. 1).

We therefore included all these significant variables in a multivariate logistic model, with all two-way interactions. Based on data from 414 cats, the selected model (AICc weight = 0.207) included the age of cats and their living environment/lifestyle to explain parasite prevalence (Table 3). We observed a significant decrease of parasite prevalence as cats aged, with 6 – 24 months old cats having a lower prevalence than  $< 6$  months old cats (OR = 0.36 [0.17, 0.75]<sub>95% CI</sub>) and a higher prevalence than  $> 24$  months old (OR = 4.20 [2.03; 9.25]<sub>95% CI</sub>). Cats with access to outdoors in city and countryside had a higher prevalence of parasites compared to cats without access to outdoors (OR = 3.89 [1.50; 9.95]<sub>95% CI</sub> and 5.34 [2.67; 11.34]<sub>95% CI</sub>, respectively), and no differences were observed between cats with access to outdoors in city or countryside ( $P = 0.453$ ).

Using a data subset including the previously selected variables in the multivariate model (age and living environment/lifestyle) and only cats  $> 6$  months old ( $n = 367$ ), we tested the influence of the neutered/spayed status on parasite prevalence. The best model included the additive effect of the reproductive status (AICc weight = 0.623) with a significantly lower parasite prevalence in neutered/spayed cats compared to intact cats (OR = 0.33 [0.14; 0.73]<sub>95% CI</sub>;  $P = 0.007$ ).

## Discussion

This study estimated the prevalence of major digestive and respiratory helminths in client-owned dogs and cats in France and explored associations between parasitic infestation and qualitative factors. The results indicate that *Toxocara cati* in cats, and *T. canis* and *Uncinaria stenocephala* in dogs, were the most common helminths detected in the faecal samples, whereas whipworms (*Trichuris vulpis*) in dogs, tapeworms (*Dipylidium caninum* and Taeniidae), and lungworms (*Angiostrongylus vasorum*, *Aelurostrongylus abstrusus*) were less commonly found. Significant correlations were observed between infection and the following criteria: age and deworming habits for dogs and cats, reproductive status,

food type, presence of other animals in the house, and living environment/lifestyle for cats, and living environment, dog's activity and time spent outside off-leash for dogs.

### Estimated parasite prevalence

Previous epidemiological surveys performed in France on the prevalence of internal parasites in pets showed contrasting results. Comparing these studies is difficult due to their different designs, methods used for detecting parasites, animal populations, geographical location, environment, age distribution and season [7, 8, 15, 16].

In the present study, 15.2% of the dogs and 14.6% of the cats were infected by at least one of the targeted parasites, which is below the prevalence rates of the last national report from 1997 (21.6% in dogs and 17.3% in cats) [8]. In both studies, the prevalence of infection in animals <1 year-old was relatively similar for dogs, but lower for cats in the present study compared to results obtained in 1997 (27.7% vs 24.7% in dogs and 31.7% vs 23.5% in cats, in 1997 and in the present study, respectively). Ascarids (*Toxocara canis* and *T. cati*) were the most frequent helminths (8.5% in dogs and 11.3% in cats) with prevalence rates similar to previous studies (5.4 – 23% for *T. canis* and 2.9% – 14.2% for *T. cati* [8, 15, 17]. We didn't detect *Toxascaris leonina* in dogs and only at a very low prevalence in cats (0.2%) as in previous French reports, with usual findings of less than 1% of positive animals [6, 7, 18]. In the present study, hookworms were identified in dogs (*A. caninum* and *U. stenocephala*; 1.7% and 4.3%, respectively), but not in cats (*A. tubaeforme*). These results were within the range of prevalence rates for these parasite species (0.5–3.4% for *A. caninum* and 2.1–17.2% for *U. stenocephala*) published in other studies conducted in France [15, 18–20]. *Trichuris vulpis* was detected in only 2.7% of the dogs in our study whereas prevalence rates in another survey in France reached 19% [18]. This difference may be due to the fact that we only enrolled client-owned dogs while in the previous surveys animals living in groups were included and also to differences of sensitivity of coproscopical methods. Prevalence rates for tapeworms based on coproscopy, generally do not exceed 3% in France [6, 7]. We detected tapeworms (*D. caninum* and Taeniidae) more frequently in cats (1.9% and 1.2%, respectively) than in dogs (*D. caninum* only [0.5%]). However, these prevalence of tapeworms might be underestimated, especially due to the intermittent rectal excretion of gravid segments.

Cardio-respiratory nematodes were rarely detected in the faeces of dogs and cats (0.5% for the French heartworm *Angiostrongylus vasorum* and 0.7% for *Aelurostrongylus abstrusus*). This prevalence of *Angiostrongylus vasorum* is lower than in previous studies in France [1.14-1.25%; 17,21,22] and in countries bordering France [e.g., 0.5 - 3.1%; 23–25] in healthy client-owned dogs, with differences according to the detection method used (antigen detection, antibody detection and/or coproscopical analyses). In France, the cat lungworm, *Aelurostrongylus abstrusus*, is considered sporadic. However in recent years, the distribution of this parasite seems to be spreading in several countries, with prevalence rates up to 20% in enzootic areas [6, 26, 27]. In our study, the other metastrongyloid, *Troglostrongylus* spp., responsible for severe respiratory disease in cats, was not detected whereas it has been recently reported in Southern Europe [28–30].

## Risk factors

Younger age is associated with a higher risk of internal parasitism, as observed in our study and previous studies [31, 32]. Certain modes of transmission (e.g., trans-placental and/or trans-mammary contamination) that are exclusive to the newly whelped or neonates and the limited immunity to parasites in young pets explain the higher prevalence of *T. canis* or *T. cati* in young individuals [6, 8].

Living environment and lifestyle are also major factors influencing parasite risk for both dogs and cats. A positive correlation between parasite prevalence and rural areas has been described for *T. cati*, *Ancylostoma* spp., and lungworms in cats [32] and *T. canis* in dogs [33]. The higher parasite prevalence in cats with outdoor access was previously reported for *T. cati* and *Ae. abstrusus* [6, 32, 33].

We observed that outdoor access, rural areas, time off-leash, and hunting/herding for dogs are the main factors increasing the risk of parasite risk, with odds-ratio up to 5.34. All these living conditions are associated with outdoor access of animals with no or limited control of animal activities from the owner when outdoors, and outdoor areas are typically larger and vegetated. In addition, wildlife is more abundant and diverse in rural and natural areas. All these factors are expected to increase the probability of encountering infective parasite stages by dogs and cats, either on the ground or in intermediate/paratenic hosts (e.g., snails, birds, rodents).

Cats living with several other pets were significantly more infected than cats living alone or with few animals and a similar trend was observed for dogs in our study. In a previous study, cats living with one or two other cats were not significantly more infected than cats living alone, but for higher densities of cat populations (more than 3 other cats in the house), the risk for *Toxocara* infestation was significantly more important [6]. The higher prevalence observed can be due to a higher risk of contamination due to the higher number of animals in a limited environment, to a higher probability to hunt and eat prey, and also, perhaps to a lower interest or financial support for veterinary care by owners of several animals.

The food type was associated with parasite prevalence in cats in univariate analyses, with a higher prevalence in cats not fed exclusively with commercial diets. The lowest prevalence was observed in cats exclusively fed commercial diets, and prevalence increases with the partial or full replacement of commercial diets. Parasite prevalence exceeded 30% in cats exclusively fed with homemade food and raw meat. While such a factor was not relevant in a previous study [34], it might partly be explained by the presence of infective parasite stages in raw or undercooked meat.

Intact cats more frequently harboured parasites in their feces than spayed/neutered cats in our study. Such findings can result from lower roaming activities in neutered/spayed cats compared to intact cats, decreasing potential risk of exposure to parasites [35], even if some authors didn't observe any difference in activity level according to the reproductive status [36, 37]. In addition, intact cats are probably less medicalized than neutered/spayed cats and, therefore, deworming is probably less frequent (never dewormed cats >6 months of age: 35.9% (n = 37/103) and 16.8% (n=44/262) of intact and neutered, respectively; P < 0.001).

## Deworming habits/recommendations

Guidelines for the control and treatment of parasites in pet animals are proposed by the European Scientific Council Companion Animal Parasite (ESCCAP) [38]. For instance, puppies should be treated with appropriate anthelmintics against roundworms from the age of 2 weeks, then every 14 days up to 2 weeks after weaning because of milk transmission, and then monthly up to 6 months of age. The schedule should be similar in cats, except that because prenatal infection does not occur in kittens, fortnightly treatment can begin at 3 weeks of age [38]. Then, the different risk factors are described to help veterinarians propose a customized deworming program to pet owners.

Although the majority of pet owners give anthelmintic drugs, our results show that most of them do not follow the ESCCAP recommendations [38]. Thirty-two percent and 23.8% of all dogs and cats, respectively, had not been dewormed within the 12 previous months. The proportion of never dewormed animals was the highest in animals less than 6 months of age ( $\leq 6$  months vs.  $>6$  months of age: 70.7% (n=41/58) vs. 22.2% (n = 81/365) for cats; 31.8% (n = 27/85) vs. 7.5% (n = 23/306) for dogs). However, young animals were often only a few months old when recruited into the study, when they were presented to the clinic for vaccines, and their owner(s) did not have any recommendation from a vet before study recruitment. This can explain the low frequency of previously dewormed animals in the young animal group. The generally advocated four-times-a-year deworming advice was poorly applied as only 38.9% (n=37/95) and 24.1% (n=81/208) of dogs and cats, respectively, older than 2 years with outdoor access receive  $\geq 3$  deworming treatments per year. Moreover, as suggested by the results of recent studies [39, 40], a significant percentage of dogs or cats could “benefit” from more frequent treatment (or fecal analyses) as suggested by ESCCAP [38].

The results obtained in this study show that animals receiving no anthelmintic treatment were significantly more infected by helminths than animals receiving 1-2 treatments/year. Surprisingly, parasite prevalence was similar in animals never dewormed and animals dewormed  $\geq 3$  times per year. These unexpected observations that have also been reported in previous studies [16] may be partially explained by possible reinfestations from a contaminated environment, possible reactivations of *Toxocara* spp. larvae encysted [41], coprophagy in dogs leading to false positive faecal samples. The significant effects of deworming frequency on parasite prevalence in univariate tests but not in multivariate analyses suggests statistical biases. Confounding effects with age cannot be excluded as young pets where often never dewormed at the first visit to the vet but dewormed monthly prior to the following examination.

## Conclusion

The results of the present study confirm that roundworms (*T. canis* and *T. cati*) are the most common helminths found in owned pets in France, and infections with other gastrointestinal nematodes such as hookworms (*U. stenocephala*, *A. caninum* and *A. tubaeforme*), whipworm (*T. vulpis*) and cestodes continue to regularly occur.

The age, reproductive status, dog's activity, living environment, lifestyle, and husbandry were risk factors associated with the helminth infection in this animal population. Furthermore, considering both the zoonotic potential of some of these parasites and the low deworming frequencies reported, veterinarians should increase awareness of pet owners and adherence to deworming guidelines based on the individual risk assessment and regular coproscopic examination. Parasite control programs could notably benefit from the implementation of a "one health approach", improving the communication between pet owners, veterinarians and physicians.

## Abbreviations

Epg

Eggs per gram

ESCCAP

European Scientific Council Companion Animal Parasites

## Declarations

### Ethics approval and consent to participate

Consent to participate provided by each pet owner.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets supporting the conclusions of this article are included within the article. Due to commercial confidentiality of the research, data not included in the manuscript can only be made available to bona fide researchers subject to a non-disclosure agreement.

### Competing interests

The authors LC, CR, JD were employees of Elanco Animal Health during the study. GB, PB, EL, BP, MPCC, EB have no competing interests.

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### Authors' contributions

LC, CR, GB, PB, EL, BP, MPCC, EB participated in one or more of design, completion and reporting of the current study. All authors participated to the redaction of the manuscript and revised it critically and

approved the final version to be published.

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## Figures

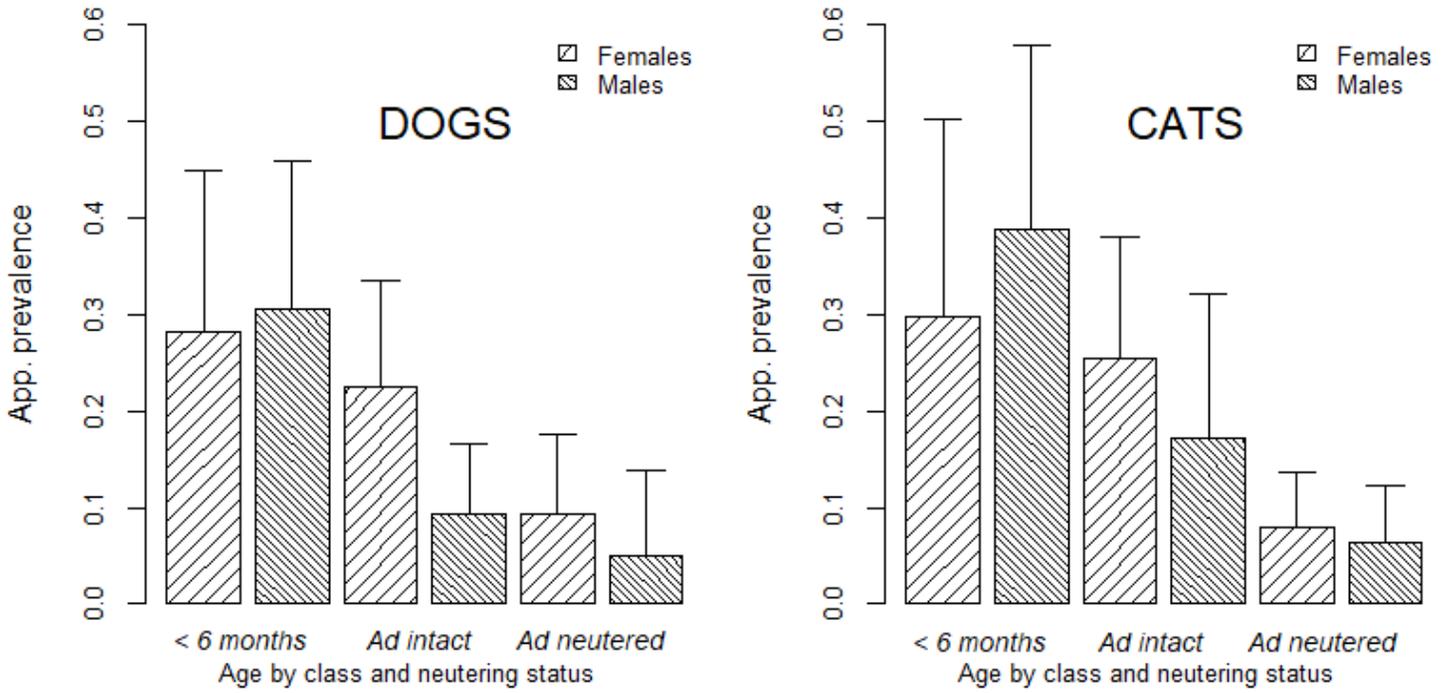


Figure 1

apparent prevalence and CI [95%] by age and reproductive status for dogs and cats

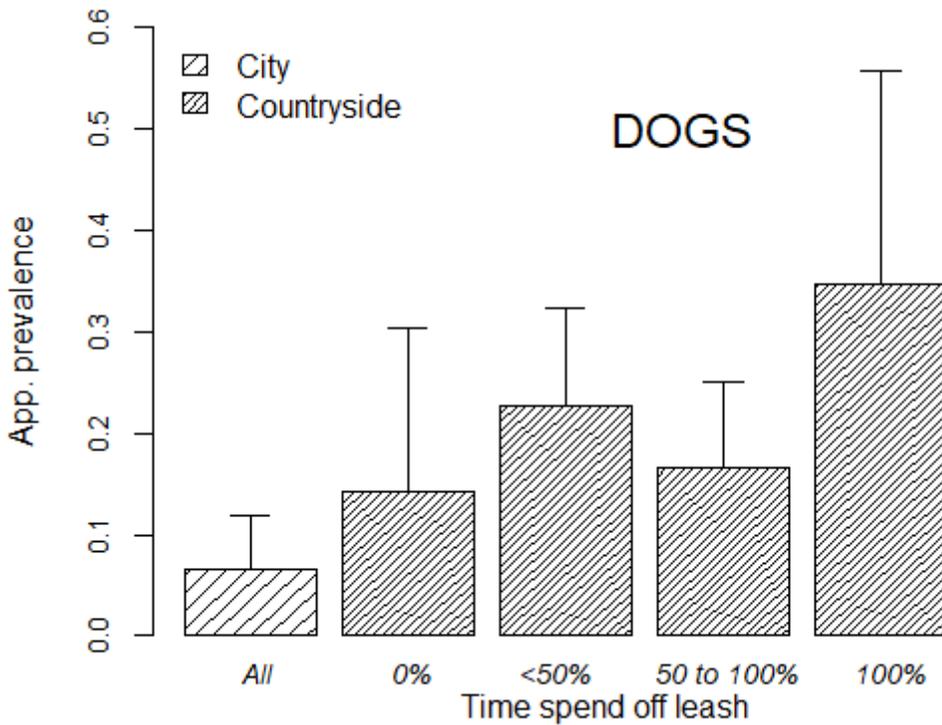


Figure 2

apparent prevalence and CI [95%] by living environment and time spend off-leash only for dogs

## Supplementary Files

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