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## Epidemiological survey on gastrointestinal and pulmonary parasites in cats around Toulouse (France)

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### Summary

Various feline parasites are potentially zoonotic thus establishing factors associated with parasitism is of animal and public health interest. The aim of this study was to determine the prevalence of endoparasites in client-owned cats, living in the area of Toulouse, France, over the period 2015 – 2017, and to investigate possible risk factors. A total of 498 faecal samples from cats of the University Animal Hospital of Ecole Nationale Vétérinaire de Toulouse were analysed, 448 from cats presented for consultation and 50 at post-mortem. Analysis was performed using a commercial flotation enrichment method with a hypersaturated sodium chloride solution and Baermann technique. Further examination of the gastrointestinal tract contents was conducted on necropsied cats. Overall, 11.6 % of cats were positive for endoparasites; 50 (11.2 %) consultation cases and 8 (16 %) post-mortem cases, with no significant difference in prevalence between the groups. Amongst infected cats, most were infected by a single species of parasite and 10.3 % (n=6) were infected with two or more. The most common parasite was *Toxocara cati* with a prevalence of 9.4 % (n=47). Other endoparasites encountered were: *Cystoisospora* sp 1.0 % (n=5), *Aelurostrongylus abstrusus* 1.0 % (n=5), *Strongyloides* sp 0.6 % (n=3), *Dipylidium caninum* 0.4 % (n=2), *Aonchotheca putorii* 0.2 % (n=1), *Ancylostomatidae* 0.2 % (n=1) and *Toxascaris leonina* 0.2 % (n=1). The examination of the gastrointestinal tract contents of the necropsied cats revealed *Mesocostoides* sp 0.4 % (n=2) and *Tænia (Hydatigera) tæniaeformis sensu lato* 0.2 % (n=1) which are seldomly diagnosed by flotation methods. In this study, increasing age and neutered status were statistically associated with reduced odds of infection by endoparasites (helminth and coccidian). Predictors of significantly increased risk included being male, intact, and not receiving regular anthelmintic treatment. The same risk factors were highlighted specifically for *Toxocara cati* infections, with rural location being an additional risk factor for infection.

**Keywords:** Cats; endoparasites; gastrointestinal; pulmonary; flotation; Baermann

### Introduction

Feline gastro-intestinal and pulmonary parasites are numerous and most of time well documented (Beugnet & Halos, 2015; Zajac

*et al.*, 2021). Infection can present as respiratory, gastrointestinal or even neurological disorders (Traversa, 2012; Andersen *et al.*, 2018; Cavalera *et al.*, 2019; Tinoco *et al.*, 2022). Clinical signs are not always present; their expression and severity are variable

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and depend on diverse factors such as parasite species, parasitic fauna and burden, immunological status of the patient, sex, or age (Duarte *et al.*, 2016). Parasitic infections are relevant to general practitioners as part of the differential diagnoses for numerous clinical presentations (Gough and Murphy, 2015). Whilst there are many inexpensive and reliable diagnostic methods available (flotation, Baermann method, SNAP tests), parasitic infections are often not diagnosed definitively, rather infection is presumed if clinical signs resolve after empirical anthelmintic treatment.

In most French households, outdoor cats have unrestricted access to their indoor environments that are shared with owners. Cats continually shed environmentally resistant infectious stages of parasites (Desplazes *et al.*, 2011; Dado *et al.*, 2012; Shapiro *et al.*, 2019), and some have a zoonotic potential, for instance: *Toxocara cati*, *Dipylidium caninum*, *Echinococcus multilocularis* or *Toxoplasma gondii* (Knapp *et al.*, 2016; Roussel *et al.*, 2019; Li *et al.*, 2022; Merigueti *et al.*, 2022; Rousseau *et al.*, 2022). Hence knowledge of the prevalence of parasitism is of importance to protect cat and human health, raise awareness and assess current worming regimes.

Despite its public health implications, the existing data regarding the distribution of feline endoparasites in France is incomplete. Indeed, the most recent information was provided by two large field studies involving 1519 and 1990 cats from 9 and 12 countries respectively; including France (Beugnet *et al.*, 2014; Giannelli *et al.*, 2017). Other data available come either from veterinary doctoral theses (Loge 2001, unpublished results; Gibier 2007, unpublished results) or from older studies with a limited number of samples (Franc *et al.*, 1997; Beugnet *et al.*, 2000).

In these French studies, and as observed in the rest of Europe, the most common parasite encountered in privately owned cats is *Toxocara cati* with prevalence ranging from 2.9 to 19.7 % (Beugnet *et al.*, 2000; Beugnet *et al.*, 2014; Overgaauw and Nijse, 2020). The second most common endoparasite varies from study to study with *Aelurostrongylus abstrusus* or *Cystoisospora* sp. regularly reported (Gibier 2007, unpublished results; Gianelli *et al.*, 2017). Other helminths such as *Strongyloides* sp., *Dipylidium caninum*, Capillariidae, *Ancylostomatidae*, *Toxascaris leonina*, *Mesocystoides* sp and *Tænia (Hydatigera) tæniaeformis sensu lato* are less frequently described with prevalence rarely exceeding a few percent (Coati *et al.*, 2003; Beugnet *et al.*, 2014; Giannelli *et al.*, 2017). Therefore, the aim of this study was to investigate the current prevalence of gastrointestinal and pulmonary helminths as well as coccidian parasites, in privately owned domestic cats in the area of Toulouse, France and to determine potential risks factors for infection.

## Material and Methods

### Sampling strategy and study area

Cats were recruited between November 2015 to February 2017; participants were either cats presented for a consultation at École

Nationale Vétérinaire de Toulouse (ENVT) (consultation group) or cats that underwent a post-mortem examination at ENVT (post-mortem group). Owners were contacted by phone on the evening prior to their appointment and asked to bring a sample of fresh stool collected from the cat's litter box. In the post-mortem group, the contents of the digestive tract were sampled by segment and separately stored (stomach, small intestine, large intestine, and rectum). The lungs and stomach were also retrieved. Most owners coming to ENVT live locally and the area of study was determined as a 75km radius area centred on Toulouse.

### Questionnaire

A paper-based questionnaire was carried out by the clinician during the consultation. Owners were asked to provide information about their pet (date of birth, sex, reproductive status, breed) and to answer multiple choice questions regarding last deworming treatment, rural or urban living environment, access to outdoor, diet and hunting habits of their cat.

### Copromicroscopic examination

Faecal samples were grossly examined for endoparasites (presence or absence of macroscopic parasitic elements). Then, a passive flotation enrichment method with a commercial kit (Ovassay Plus kit<sup>ND</sup>, Zoetis, Malakoff, France) was used according to the manufacturer recommendation. Specifically, 2 grams of fresh faeces were mixed with a hypersaturated sodium chloride solution (specific gravity [sg] of 1.2) and examined after 10 minutes microscopically (Zeiss Primostar, Carl Zeiss France S.A.S.). Finally, helmintho-larvoscopy was performed using the Baermann method. Parasites, oocysts, and eggs were identified using morphological keys (Beugnet and Halos, 2015; Traversa and Di Cesare, 2016; Morelli *et al.*, 2021; Zajac *et al.*, 2021).

### Investigation of parasites in post-mortem group

After dilution in lukewarm water, the stomach and small intestines contents were examined under a dissecting microscope (Zeiss Stemi 305, Carl Zeiss France S.A.S., Marly le Roi, France). A scrape of gastric mucosa was also acquired to identify parasites and eggs, both under dissecting microscope and microscope. A modified Baermann method was used to further assess the presence of pulmonary parasites. To this end, faeces were replaced by one-centimeter cubes of lungs. Copromicroscopic analysis was also performed, as previously described, on stool samples collected directly from the rectum.

### Statistical analysis

Prevalence (p) was defined as the percentage (number of positive results / number of individuals in the considered population (n)) and the corresponding 95 % confidence interval as  $(p \pm 1,96 \sqrt{(p(1-p))}) / \sqrt{(n)}$ . Prevalence was compared between groups using the Fisher's Exact test. The association between parasitism and variables of interest was assessed using univariable and multi-

Table 1. Prevalence of parasitism, for a single species or more endoparasites (CI: 95% Confidence Intervals) stratified according to group. Consultation group represent cats presenting for consultation and postmortem group, cats for which parasites were identified at post-mortem.

	Consultation group (n=448)	Postmortem group (n=50)	Total (n=498)
Positive, n	50	8	58
% (CI)	11.2 (8.2 – 14.1)	16 (5.8 – 26.2)	11.6 (8.8 – 14.5)
Of these			
One parasite species, n	48	4	52
% (CI)	96.0 (94.2 – 97.8)	50 (36.1 – 63.9)	89.7 (87.0 – 92.3)
Two parasite species or more, n	2	4	6
% (CI)	4.0 (2.2 – 5.8)	50 (36.1 – 63.9)	10.3 (7.7 – 13.0)

variable logistic regression analysis. Odds ratios (ORs) and their 95 % confidence intervals (CIs) for positivity are also presented. Variables with  $p < 0.2$  in the univariable analyses were included in a backwards, stepwise, multivariable logistic regression model. Statistical significance was defined as a  $p$ -value of  $< 0.05$ . Statistical analyses were performed by computerized statistical software (SPSS 27.0 for Windows, SPSS Inc, Chicago, IL).

### Ethical Approval and/or Informed Consent

Informed consent was obtained from the owners of all the patients included in this study. All applicable national and institutional guidelines for the care and use of animals were followed.

### Results

#### Prevalence of parasitism

Overall, 11.6 % (58/498) of the samples were positive for endoparasites, with 11.2 % of the consultation group (50/448) and 16.0 % of the post-mortem group (8/50) being positive for parasites. There was no significant difference in prevalence of parasitism between the groups ( $P=0.35$ ). Among infected cats, most were infected by a single species of parasite and only 10.3 % of cases (6/58) harboured two or more species (Table 1). Mixed infections were significantly more frequent in the post-mortem group than the consultation group ( $P=0.002$ ).

*Toxocara cati* was predominant and found in 9.4 % of cats (47/498) (Table 2). An overall prevalence of 1.0 % (5/498) was found for both *Cystoisospora* sp, identified by flotation, and *A. abstrusus*, identified with the Baermann method. First stage larvae of *A. abstrusus* were identified in the faeces of 4 feline patients and in both faeces and lungs of a necropsied cat. Other parasites were also detected but with lower prevalence. *Strongyloides* sp larvae were observed using the Baermann method in 3 cats (0.6 %). The prevalence of *Dipylidium caninum* was 0.4 %, with egg packets

identified on flotation in the faeces of 2 individuals. *Toxascaris leonina* eggs were found in a single patient cat (0.2 %). Finally, some endoparasites were only retrieved from necropsied cats. Adult parasites were directly observed within the digestive tract: *Mesocostoides* sp ( $n=2$ , overall prevalence 0.4 % and *Taenia (Hydatigera) taeniaeformis sensu lato* ( $n=1$ , overall prevalence 0.2 %). Eggs of *Ancylostomatidae* and *Capillariidae* were also observed on flotation in one individual each. Regarding *Capillariidae*, the adults were found in the scraped stomach mucosa and identified as *Aonchotheca putorii*.

#### Determination of risk factors

##### Overall parasitism

Prevalence of endoparasites stratified by putative risk factors is presented in Table 3. Univariable logistic regression identified increasing age and overall neutered status (males and females combined) as associated with reduced odds of parasitism (Table 4). Risk factors associated with increased risk of parasitism included being male, being entire (male and female combined) and having never received anthelmintics or anthelmintic treatment more than 6 months ago. When considering sex and reproductive status together, neutered females and neutered males had reduced risk of parasitism whilst entire males had a significantly increased risk for parasitism.

Multivariable logistic regression analysis revealed that increasing age (OR 0.899 [95 % CI 0.82–0.96];  $P=0.024$ ), and anthelmintic administration in the past 6 months (OR 0.336 [95 % CI 0.182–0.613];  $P<0.001$ ) were independently associated with decreased risk of overall parasitism, whereas rural location (OR 2.497 [95 % CI 1.117–5.580];  $P=0.026$ ) and entire male status (OR 3.028 [95 % CI 1.652–5.547];  $P<0.001$ ) were independently associated with increased risk of overall parasitism. The Hosmer-Lemeshow goodness of fit test confirmed good model fit ( $P=0.613$ ). Neutered females and neutered males were not at significantly reduced risk of overall parasitism after adjustment for other factors.

Table 2. Types of endoparasites identified, by decreasing frequency and stratified by group. Consultation group represent cats presenting for consultation and postmortem group, cats for which parasites were identified at post-mortem.

	Consultation group (n=448)		Postmortem group (n=50)		Total (n=498)	
<i>Toxocara cati</i> , n	40		7		47	
% (CI)	8.9	(6.3 – 11.6)	14	(4.4 – 23.6)	9.4	(6.9 – 12.0)
<i>Cystoisospora</i> sp, n	5		0		5	
% (CI)	1.1	(0.1 – 2.1)	0		1.0	(0.1 – 1.9)
<i>Aelurostrongylus abstrusus</i> , n	4		1		5	
% (CI)	0.9	(0 – 1.8)	2	(0 – 5.9)	1.0	(0.1 – 1.9)
<i>Strongyloides</i> sp, n	2		1		3	
% (CI)	0.5	(0 – 1.1)	2	(0 – 5.9)	0.6	(0 – 1.3)
<i>Mesocestoides</i> sp, n	0		2		2	
% (CI)	0		4	(0 – 9.4)	0.4	(0 – 1.0)
<i>Dipylidium caninum</i> , n	1		1		2	
% (CI)	0.2	(0 – 0.7)	2	(0 – 5.9)	0.4	(0 – 1.0)
<i>Tænia (Hydatigera) tæniaeformis</i> <i>sensu lato</i> , n	0		1		1	
% (CI)	0		2	(0 – 5.9)	0.2	(0 – 0.6)
Capillariidae, n	0		1		1	
% (CI)	0		2	(0 – 5.9)	0.2	(0 – 0.6)
Ancylostomatidae, n	0		1		1	
% (CI)	0		2	(0 – 5.9)	0.2	(0 – 0.6)
<i>Toxascaris leonina</i> , n	1		0		1	
% (CI)	0.2	(0 – 0.7)	0		0.2	(0 – 0.6)

### *Toxocara cati*

Prevalence for *T. cati* stratified by putative risk factors is presented in Table 5. Univariable logistic regression analysis revealed similar risk factors as for overall parasitism (Table 4). When considering sex and reproductive status together, male entire was the only status associated with an increased risk for infection. Neutered males and neutered females were not significantly less likely to have *Toxocara cati*.

Multivariable logistic regression analysis revealed that increasing age (OR 0.876 [95 % CI 0.786–0.975]; P=0.015), and anthelmintic administration in the past 6 months (OR 0.221 [95 % CI 0.112–0.438]; P<0.001) were independently associated with decreased risk of *Toxocara cati* infection whereas rural location (OR 2.497 [95 % CI 1.117–5.580]; P=0.026) and entire male status (OR 3.250 [95 % CI 1.130–7.712]; P=0.008) were independently associated with increased risk of *Toxocara cati* infection. The Hosmer-Lemeshow goodness of fit test confirmed good model fit (P=0.458).

### Discussion

The present study, through the analysis of 498 faecal samples obtained from both living individuals and necropsied cats with

various background and lifestyle, represents one of the most extensive parasitological studies conducted in France over the last 20 years. Indeed, previous ones involved either a smaller sample population, unpublished data from veterinary doctoral theses or, for those conducted on a larger scale, a lack of information on the prevalence among the samples coming from French felines (Table 6). Additionally, prevalence of endoparasites in cats obtained through *post-mortem* examination has, to the author's knowledge, never been reported in France; the main interest of this method being to limit the risk of false negatives related to intermittent or scarce shedding of eggs and parasitic elements. This increased sensitivity likely contributes to the significantly higher prevalence of mixed infections observed in the *post-mortem* group. Based on copromicroscopy alone, 50 % (2/4) of the individuals with mixed infections would have been misdiagnosed as harbouring a single parasite. Similarly, 37.5 % (3/8) of positive individuals would have been considered negative for parasites.

With an overall infection rate of 11.8 % among privately owned cats from the area of Toulouse, the prevalence is remarkably close to the one obtained 10 years ago in the same area on a similar population (23/202, prevalence of 11.4 %, Gibier 2007, unpublished data, 2007). It is equally reasonably close to the 17.3 %

Table 3. Endoparasite prevalence of consultation group (cats presenting for consultation) and postmortem group stratified by various factors.

	Consultation group			Postmortem group			TOTAL		
	Total headcount	Positive	Prevalence	Total headcount	Positive	Prevalence	Total headcount	Positive	Prevalence
<b>TOTAL</b>	448	50	11.2	50	8	16.0	498	58	11.6
<b>SEX</b>									
Female	233	18	7.7	23	3	13.0	256	21	8.2
> Entire	140	14	10.0	3	1	33.3	143	15	10.5
> Neutered	93	4	4.3	20	2	10.0	113	6	5.3
Male	215	32	14.9	27	5	18.5	242	37	15.3°
> Entire	136	31	22.8	10	2	20.0	146	33	22.6
> Neutered	79	1	1.3	17	3	17.6	80	4	5.0
<b>REPRODUCTIVE STATUS</b>									
Neutered	172	5	2.9	37	5	13.5	209	10	4.8
Entire	276	45	16.3	13	3	23.1	289	48	16.6°
<b>LIFESTYLE</b>									
inside strictly	235	20	8.5	16	3	18.8	251	23	9.2
access to outdoor	212	30	14.2	23	4	17.4	235	34	14.5
<b>LIVING ENVIRONMENT</b>									
rural	47	8	17.0	9	2	22.2	56	10	17.9
urban	401	42	10.5	41	6	14.6	442	48	10.9
<b>HOUSEHOLD</b>									
alone	273	27	9.9	21	3	14.3	294	30	10.2
other animals	171	23	13.5	14	5	35.7	185	28	15.1
<b>DIET</b>									
hunter	53	8	15.1°	ND	ND	ND	ND	ND	ND
not hunting	395	42	10.6	ND	ND	ND	ND	ND	ND
<b>TIME SINCE LAST WORMER</b>									
<6 months	290	21	7.2	16	4	25.0	306	25	8.2
never or >6months	129	27	20.9	14	0	0.0	143	27	18.9°
<b>AGE</b>									
< 6 months	124	29	23.4	1	1	100.0	125	30	24.0
> 6 months	324	21	6.5	47	7	14.9	371	28	7.5°

° significant difference among categories (P<0.05), ND : no data

reported in a study conducted on 98 patient cats from the 4 French veterinary faculties in 1997 (Franc *et al.* 1997) and the 8.8 % obtained in the area of Paris in 2000 among 34 privately owned cats (Beugnet *et al.*, 2000). Nevertheless, this prevalence is markedly lower to the ones reported in the 2 largest European studies in which France took part with respectively 35.1 % (Beugnet *et al.*, 2014) and 30.8 % (Giannelli *et al.* 2017).

A potential explanation could be that the methods of detection were different. Indeed, in the study by Beugnet *et al.* (Beugnet *et al.*, 2014), a centrifugal flotation technique derived from the Stoll method was used. However, Gianelli and others (Giannelli *et al.* 2017) elected the McMaster method which, apart from being quantitative when the technique used in the current study is only semi-quantitative, relies on the same flotation principle. Similarly, although

the solutions employed in these two studies were varied and included zinc sulphate (sg=1.2), zinc sulphate + acetate (sg=1.33), sodium chloride (sg=1.2) or sucrose solution (sg=1.2); they had similar specific gravities as in the present one. Salt flotation, despite having some limitations for detection of some eggs such as trematode or cestode eggs and *Giardia* cysts, is inexpensive, easily replicable and among the most frequent methods used by practitioners. Comparison between methods is difficult but Alcaïno and Baker (Alcaïno and Baker, 1974) showed little difference, from a qualitative point of view (presence/absence of parasitic elements) between direct centrifugal flotation and gravitational flotation. Nevertheless, the parasitic burden was likely underestimated in the present study, owing to the use of simple gravitational flotation. This remains acceptable since quantification of shedding was not one of the objectives.

An additional explanation regarding the difference in prevalence between France and Europe could be the influence on the overall result of higher ongoing prevalence in some of the countries surveyed. In Italy, infection rates in domestic cats have been reported

as high as 60 % in the past (Zanzani *et al.*, 2014) and close to 36 % more recently (Genchi *et al.*, 2021). In Hungary, a study on 235 domestic cats reported a prevalence of 40 % (Capari *et al.*, 2013). In Romania, reported prevalence range from 13.8 % in a cohort of 58 cats both privately owned or from shelters (Sorani *et al.*, 2015) to 34 % in a study investigating 414 household cats (Mircean *et al.*, 2010). Finally, 16.5 % of the 103 household cats surveyed in a nationwide study in Spain were positive for intestinal parasites (Miro *et al.*, 2004).

*Toxocara cati* was the most frequently encountered parasite in this study with a prevalence of 9 %, but also throughout Europe (Beugnet *et al.*, 2014; Gianelli *et al.*, 2017; Overgaauw and Nijse, 2020). Reported infection rates by *T. cati* in domestic cats are highly variable, ranging from 3.9 % in Germany (Raue *et al.*, 2017) to 40 % in Romania (Ursache *et al.*, 2021) but remain largely inferior to those reported in stray cats, that are regularly above 45 % (Knaus *et al.*, 2014; Takeuchi-Storm *et al.*, 2015; Zottler *et al.*, 2019). Nevertheless, this result is similar to the one obtained in 2006 in the same area (overall prevalence 9 %) (Gibier 2007,

Table 4. Univariable analysis of factors significantly associated with positive result for overall parasitism or for *Toxocara cati* specifically.

	n	sig	OR	95% CI for OR	
<b>Overall parasitism</b>					
Age (years)	496	0.003	0.87	0.79	0.95
Sex (male vs. female [referent])	498	0.015	2.02	1.15	3.56
Reproductive status (neutered vs. entire [referent])	498	<0.001	0.25	0.12	0.51
Anthelmintic treatment (administered in last 6 months vs. never administered or >6 months ago [referent])	449	0.001	0.38	0.21	0.69
Breed (purebred vs. non-purebred [referent])	498	0.175	0.37	0.09	1.56
Location (rural vs. urban [referent])	497	0.130	1.78	0.84	3.76
<i>Sex and reproductive status</i>					
> Neutered females (vs. entire females and all males combined [referent])	113	0.021	0.36	0.15	0.86
> Entire males (vs. neutered males and all females combined [referent])	146	<0.001	3.82	2.18	6.7
> Neutered males (vs. entire males and all females combined [referent])	96	0.017	0.28	0.1	0.79
<b><i>Toxocara cati</i></b>					
Age (years)	47	0.004	0.85	0.76	0.95
Sex (male vs. female [referent])	47	0.03	1.99	1.07	3.71
Reproductive status (neutered vs. entire [referent])	47	0.004	0.34	0.17	0.71
Anthelmintic treatment (administered in last 6 months vs. never wormed or >6 months ago [referent])	43	<0.001	0.27	0.14	0.51
Breed (purebred vs. non-purebred [referent])	47	0.143	0.22	0.3	1.66
Location (rural vs. urban [referent])	47	0.078	2.03	0.93	4.46
<i>Sex and reproductive status</i>					
> Entire males (vs. neutered males and all females combined [referent])	26	<0.001	3.42	1.85	6.3



unpublished results). It is equally close to the prevalence inferred from 2 French veterinary faculties included in a global European study (Beugnet *et al.* 2014). Indeed, in this project conducted in 2014, faecal samples of patient cats from the University Teaching Hospitals of Nantes and Maisons-Alfort were analysed. *Toxocara cati* was found in 11/91 and 6/96 samples from the aforementioned institutions, respectively, representing an overall prevalence in French centres of 9 % (17/187). Prevalence previously reported in France was slightly higher; 14 % among 180 patient cats from all four French veterinary faculties (Franc *et al.*, 1997), 14 % in 34 domestic cats in 2000 (Loge 2001, unpublished results) and 11 % in a study conducted on 3000 cats from France and Germany (Coati *et al.*, 2003).

In the present study, age was associated with parasitism in general and for *Toxocara cati* specifically. The prevalence of parasites was significantly higher in cats aged of less than 6 months than in

cats aged over 6 months, which has been noted several times in literature (Becker *et al.*, 2012; Mugnaini *et al.*, 2012; Riggio *et al.*, 2013; Capari *et al.*, 2013; Zottler *et al.*, 2019; Genchi *et al.*, 2021). It could be explained by the immature immune system of younger animals, by the communal lifestyle of kittens, or be related to a lack of anthelmintic treatment in young animals. In France, anthelmintic treatment is often given after the initial vaccination consultation which sometimes does not occur before 3 to 6 months old, and may result in young individuals being administered anthelmintics less frequently.

For both overall parasitism and *T. cati* infections, significant differences were observed between male and female and between entire and neutered individuals. In this study, females were significantly less likely to be infected than males. Several studies report no sex difference regarding the risk of infection (Mircean *et al.*, 2010; Capari *et al.*, 2013; Giannelli *et al.*, 2017) and some report males

Table 5. Prevalence of *Toxocara cati* stratified by various factors.

	n, cats positive for <i>T. cati</i>	Overall population	Prevalence
<b>SEX</b>			
Female	17	256	6.6
Male	30	242	12.4°
<b>REPRODUCTIVE STATUS</b>			
Neutered	10	209	4.8
Entire	37	289	12.8°
<b>LIFESTYLE</b>			
inside strictly	18	251	7.2
access to outdoor	28	235	11.9
<b>LIVING ENVIRONMENT</b>			
rural	9	56	16.1°
urban	38	442	8.6
<b>HOUSEHOLD</b>			
alone	24	294	8.2
other animals	23	185	12.4
<b>DIET</b>			
hunter	8	53	15.1
not hunting	32	395	8.1
<b>TIME SINCE LAST WORMER</b>			
< 6 months	17	306	5.6
never or > 6 months	26	143	18.2°
<b>AGE</b>			
< 6 months	24	123	19.5°
> 6 months	23	375	6.2
<b>TOTAL</b>	<b>47</b>	<b>498</b>	<b>9.4</b>

° significant difference among categories (P<0.05)

Table 6. Prevalence of endoparasites in cats from France, chronological order (most relevant list).

Author	Publication	Date	Area	Population	Method	Results
Franc <i>et al.</i>	Rev med vet	1997	4 National Veterinary Schools (Nantes, Paris, Toulouse, Lyon)	98 privately owned patient cats	Flotation (Ovassay <sup>TD</sup> )	17.3 % overall prevalence 14.2 % <i>T. cati</i> 3 % <i>D. caninum</i>
Beugnet <i>et al.</i>	Rev med vet	2000	Paris area	34 privately owned cats	Flotation and sedimentation (Telemann-Rivas)	8.8 % overall prevalence 2.9 % <i>T. cati</i> 2.9 % <i>Tænia (Hydatigera) tæniaeformis sensu lato</i> 2.9 % <i>Cystoisospora sp</i>
Loge	Unpublished (veterinary doctoral theses)	2001	North west of France	180 cats	ND	14 % <i>T. cati</i> 5.5 % Tapeworms
Coati <i>et al.</i>	Parasitol Res	2003	France and Germany	3500 cats	Modified McMaster method and flotation	11.3 % <i>T. cati</i> 0.4 % <i>Ancylostomatidae</i> 0.3 % <i>T. leonina</i> 0.3 % <i>D. caninum</i>
Gibier	Unpublished (veterinary doctoral theses)	2007	Toulouse area	202 privately owned patient cats	Flotation (Ovassay <sup>TD</sup> )	11.4 % Overall prevalence 9.4 % <i>T. cati</i> 1.5 % <i>Cystoisospora sp</i> 0.5 % <i>A. abstrusus</i>
Beugnet <i>et al.</i>	Parasites and vectors	2014	Several European countries including 2 French veterinary faculties (Nantes and Maison-Alfort)	1519 domestic cats but 187 from France	Flotation and Baermann method	35.1 % overall prevalence 19.7 % <i>T. cati</i> (9.1 % in French cats) 1.4 % <i>Ancylostomatidae</i> 0.3 % <i>T. leonina</i> 3.0 % <i>D. caninum</i> 4.1 % <i>A. abstrusus</i> 1.1 % Capillariidae 9.7 % <i>Cystoisospora sp</i>
Gianelli <i>et al.</i>	International Journal of Parasitology	2017	12 European countries	1990 domestic cats but 92 from France	McMaster and Baermann method	30.8 % overall prevalence 16.5 % ascarids 10.6 % lungworms 6.5 % coccidian 4.5 % hookworms

ND: no data, TD: trade name

as being significantly less likely to be infected (Zottler *et al.*, 2019). Neutering status was also associated with prevalence; entire cats were significantly more likely to be infected than neutered ones. A potential explanation could be an over representation of young individuals among the entire cats (40 % aged less than 6 months). However, entire male status remained an independent risk factor even after adjustment for age in the multivariable analysis. Entire cats with access to the outdoors could have a wider territory than neutered cats, which in turn exposes them to more sources of infection (for example by hunting rodents which are sources of hypobiotic *T. cati* larvae). Similarly, in France, cats are usually neutered around 6 months old, corresponding to the acquisition of immunity against *T. cati* and the switch to the somatic life cycle. In this study, the lifestyle of cats (indoor vs. outdoor) was not sig-

nificantly associated with parasitic prevalence. These findings conflict with the results of several recent studies in which outdoor access has been reported as a risk factor; recently a global meta-analysis comparing the risk between indoor vs outdoor cat showed that outdoor cats were 2.77 times more likely to be infected (Chalkowski *et al.*, 2019). This result highlights the importance of environmental contamination (Afonso *et al.*, 2013; Pezeshki *et al.*, 2017). Increased prevalence of *T. cati* infection in outdoor cats was also demonstrated in a large-scale European study (Beugnet *et al.*, 2014) and another recent study (Ursache *et al.*, 2021). One possible explanation would be that owners underestimate the impact of unsupervised outdoor time, and some cats may have been falsely reported as being strictly indoor when they had access to an enclosed garden or a balcony. According to a retrospective



study conducted in the Netherlands, even if cats spend less than an hour a day outdoor, they already present an increased risk for *T. cati* contamination (Nijse *et al.*, 2016). Regarding the other factors studied here such as the presence of other animals in the household or hunting habits, they were not significantly associated with the prevalence of parasitism. Similar conclusions were drawn for *T. cati* in a large European study where only higher densities of cats, or household of 3 or more cats were at increased risk of infection (Beugnet *et al.*, 2014).

Anthelmintic treatment, as expected, was also significantly associated with the prevalence of parasitism with cats that never received anthelmintics or those treated more than 6 months prior being significantly more likely to be infected than cats treated within the last 6 months, which concurs with the results of a recent Hungarian study (Capari *et al.*, 2013). Furthermore, a study carried out in 2014 in Europe showed that cats wormed 3 times a year or less were still significantly more likely to be infected than those receiving more than 3 treatments per year, thus illustrating that an adequate protective effect of worming is only obtained above a certain frequency (Beugnet *et al.*, 2014). It would have been interesting to consider the frequency of worming treatment instead of the date of last administration in the present study, although unfortunately this data was not available.

Regarding parasites other than *T. cati*, the prevalence was generally low ( $\leq 1\%$ ). *Cystoisospora* sp. was found in five cats, four of which were aged less than a year. Although the sample size was too small to perform any statistical comparisons, this reflects the significantly higher infection rates previously reported in young cats by Barutzki and Schaper (Barutzki and Schaper, 2011). Similarly, the five cases of *A. abstrusus* infection observed in the present study were in cats aged less than 8 months. Although risk factors could not be statistically determined, recent studies have shown young animals to be more at risk of infection (Cavalera *et al.*, 2019; Gueldner *et al.*, 2019; Kiszely *et al.*, 2019; Traversa *et al.*, 2019). The prevalence of *A. abstrusus* in the present study (1%) is within the wide range reported by Beugnet *et al.* (Beugnet *et al.*, 2014) (0.8 to 35.8%) but below the one reported by Gianelli *et al.* in France (Gianelli *et al.*, 2017). Indeed, in the latter study, 92 samples from cats of the area of Lyon were analysed and 4 of them proved to be positive representing a prevalence of 4.4%. In Europe, prevalence of *A. abstrusus*, obtained from Baermann examination, in domestic cats, varies greatly. Values range from less than 0.01% in Sweden (Grandi *et al.*, 2017), 0.02% in Slovakia (Šmigová *et al.*, 2021), 0.7% in Switzerland (Zottler *et al.*, 2019), to 14.3% in Sardinia (Tamponi *et al.*, 2017), 19.8% in Hungary (Kiszely *et al.*, 2019), or even 35.8% in Bulgaria (Gianelli *et al.*, 2017). It appears uneven, with endemic foci concentrated around the Mediterranean region. However, the presence of *A. abstrusus* is increasingly reported, even in areas previously considered at a low risk or unaffected (Di Cesare *et al.*, 2019). A recent study on domestic cats in Switzerland revealed a seroprevalence for *A. abstrusus* close to 10.7% (Gueldner *et al.*, 2019) when previous

studies based on the Baermann method had only evidenced prevalence of 0.8 – 2.3% (Gianelli *et al.*, 2017; Zottler *et al.*, 2019). Similarly, in the United Kingdom, country deemed unaffected based on the work of Gianelli and others (Gianelli *et al.*, 2017), a national study including both privately owned and stray cats revealed a prevalence of 1.7% (Elsheikha *et al.*, 2019). This could be a consequence of the increasing interest in feline lungworms observed over the last decade or reflect a spread of the parasite, potentially facilitated by global warming (Traversa and Di Cesare, 2013; Elsheikha *et al.*, 2016). An additional key factor is the method of detection. Although the current gold standard remains the observation of L1 larvae on Baermann examination, intermittent excretion can lead to false negative (Elsheikha *et al.*, 2016). Several recent studies involving both standard faecal examination and serological tests or molecular methods of detection suggest that feline exposition and infection could be largely underestimated (Di Cesare *et al.*, 2019; Morelli *et al.*, 2020; Morelli *et al.*, 2022; Raue *et al.*, 2021; Schnyder *et al.*, 2021). In experimentally infected cats, seropositivity has been observed in the complete absence of shedding (Raue *et al.*, 2021). In Italy, up to a fifth of randomly selected cats, negative on faecal examination with the Baermann method, were positive for *A. abstrusus* antibodies (Di Cesare *et al.*, 2019). A similar observation was made in Greece where only a third of the samples found positive on serology were also positive on Baermann (Morelli *et al.*, 2020). As a result, the prevalence obtained in the present study for *A. abstrusus* was potentially underestimated. Additional serological testing of the patients or molecular testing on the faeces would have been valuable.

*Strongyloides* sp. larvae were found in the stool of 3 cats, representing a low prevalence. In Europe, the prevalence for *Strongyloides* sp. appears relatively low: 3.4% in Romania (Mircean *et al.*, 2010) and 1% in Denmark (Takeuchi-Storm *et al.*, 2015). However, the actual prevalence of *Strongyloides* sp. in France and worldwide is largely unknown. Indeed, flotation methods are used routinely despite their known low sensitivity, whereas the use of the Baermann method should be preferred (Wulcan *et al.*, 2019). This is particularly regrettable considering its zoonotic potential. Another element explaining the lack of detection is that symptomatic infections by *Strongyloides* sp. are rare and mostly concern young individuals (Thamsborg *et al.*, 2017).

*Mesocestoides* sp., *Dipylidium caninum*, *Taenia (Hydatigera) taeniaeformis sensu lato*, *Capillariidae*, *Ancylostomatidae* and *Toxascaris leonina* were rarely identified ( $< 1\%$ ). These results are consistent with those reported in 2003 in a multicenter study from France and Germany, where the infection rates for these parasites were between 0.3–0.5% (Coati *et al.*, 2003). In contrary, the work from Beugnet and others (Beugnet *et al.*, 2014) reported a prevalence of 0% for *Ancylostomatidae* and *Toxascaris leonina* in 96 and 91 patient cats included from the French Veterinary Schools of Maison-Alfort and Nantes. Recent infection rates are not available from France but in a study conducted in Italy, prevalence of *Toxascaris leonina* and *Capillariidae* were 0.2 and 0.4% respectively,

whereas *Ancylostomatidae* were encountered in 4.9 % of samples (Traversa *et al.*, 2019). Whilst considered rare in Europe, these parasites should not be neglected, in particular *Ancylostomatidae* whose prevalence in some countries is close to 10 %: 9.9 % in Italy (Genchi *et al.*, 2021), 10.1 % in Romania (Mircean *et al.*, 2010), 11.1 % in Hungary (Capari *et al.*, 2013)). Moreover, the low prevalence observed for some parasites must be contextualised according to the detection method used. It is well known that copromicroscopy has a poor sensitivity for parasites such as *D. caninum*, because proglottids are mobile and often shed between defecation (Boreham and Boreham, 1990; Bourdeau *et al.*, 1993). In the present study, parasitic elements were spotted either in the transportation box or in the hairs of the perianal area of several cats, but the result of their copromicroscopic examination was incorrectly reported as negative (data not shown). Hence, the prevalence of proglottid parasites obtained from *post-mortem* (2 %) is much more likely to reflect the true prevalence. The same issue can be raised regarding *Mesocestoides* sp. which shed proglottids intermittently, and that are known to move away from faeces not long after emission (Loos-Frank, 1987). Thus, the prevalence of *Mesocestoides* sp. in domestic cats in the area of Toulouse is more likely to be 4 % (value obtained from the *post-mortem* cohort), rather than 0.4 % (overall prevalence).

## Conclusion

The results of this study highlight that despite simple ways to diagnose, treat and prevent parasitism, this remains common in privately-owned cats, with greater than 1 in 10 cats infected by at least one parasite. Increasing age and neutered status were associated with reduced odds of parasitism whereas being male, intact, and not receiving regular anthelmintic treatment were associated with an increased risk, which may provide further information as to the underlying pathophysiology of parasitism in cats. The analysis of the digestive contents of 50 necropsied cats allowed diagnosis of parasites that are seldomly diagnosed with standard copromicroscopic methods and gave valuable information on their current prevalence in the area. Since some of the parasites identified have a zoonotic potential, these findings further validate the current recommendations in terms of anthelmintic therapy, especially monthly treatment in young cats. Promoting regular parasite control in cats remains essential, with rational deworming including regular copromicroscopic examination as recommended by the European Scientific Counsel for Companion Animals Parasites (ESCCAP).

## Conflict of Interest

Authors have no potential conflict of interest pertaining to this submission to *Helminthologia*. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors

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