

# Multicentre Study of Endo-Ectoparasite Infection in Italian Cats

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# Abstract

## Background

Parasites infecting cats include protozoa, helminths and arthropods. Many are also transmissible to humans. Effective control relies on knowledge of parasite distribution and risk factors for infection. The present study was aimed at evaluating prevalence of major feline parasites in Italy and risk factors associated with their occurrence.

## Methods

Over a 12-month study period, thirteen study centers across Italy analyzed feces, hair and ectoparasites from naturally infected cats coming from feral colonies, shelters and private households. Samples from cats (n=987) were analyzed by all centers using the same diagnostic methods. Prevalence values and risk factors were evaluated statistically for identification of predictors of risk.

## Results

The overall prevalence of gastro-intestinal (GI) and bronco-pulmonary (BP) nematodes was 35.9% (354/987). *T. cati* was the most prevalent species (253/987; 25.6%), followed by hookworms (98/987; 9.9%). Among BP nematodes, *A. abstrusus* was the most common (76/987; 7.7%). Approximately 35.7% (352/987) of the study population was infested by ectoparasites. The most common were fleas 29.4% (290/987), followed by ear mites *O. cynotis* 9.8% (97/987).

Predictors of risk for parasite infection included age, a predominantly or exclusively outdoor lifestyle, geographic area and lack of anti-parasitic treatment.

## Conclusion

Both ecto- and endoparasites are still common in cats throughout Italy, many of them being of zoonotic concern and vectors of pathogens to humans. Given the presence of parasites throughout the entire study period, year-round treatment should be considered. Furthermore, data confirm the need to protect the human-animal bond using proper endo- and ecto-parasiticides to reduce the risk of human infection, in application of the One-Health concept.

## Background

Numerous parasites infect domestic cats. Among these, helminths of the gastro-intestinal tract and respiratory system can cause severe disease if parasite loads are heavy, while different arthropods can cause skin disease and allergy. Subclinical infection is of equal concern given the zoonotic nature of several feline helminths and the capacity of fleas and ticks to transmit pathogens to cats, other animals and humans. Recent multicenter studies in both Europe and Italy, have been carried out to define the current status of endo- and ectoparasite infections of cats [1–4]. According to these, infections are widespread and depend on various risk factors, including lifestyle, geographical area and frequency of anti-parasitic treatment. Most studies on prevalence, distribution and risk factors for feline parasites in Italy have been carried out in central and southern areas of the country [5–6].

Multicenter studies provide useful information on distribution and risk of parasite infection. However, few apply the same, standard diagnostic protocols for each center, thus compromising reliability of results. Indeed, it has been reported that different copro-microscopic techniques have differing sensitivity, specificity and accuracy for the diagnosis of gastro-intestinal (GI) and bronco-pulmonary (BP) nematodes. The Mini-FLOTAC technique [7] has been recently demonstrated to be a highly sensitive method for diagnosing parasitic infections of human and veterinary importance where larvae or ova of parasites are shed in the faeces [8–11].

Seasonality of parasite infection in cats has been evaluated mostly from retrospective, longitudinal studies [12–13]. It is possible that sampling and analysis over a fixed period of time may give more useful information on the current effects of season on parasite prevalence.

The aims of the present multicenter study were therefore to: (i) determine the current prevalence of feline endo and –ectoparasites throughout Italy by recruitment of cats from all regions; (ii) evaluate seasonal trends by recruiting a set number of cats each month

consecutively over a 12-month period; (iii) use standardized diagnostic methods in order to eliminate variables associated with differences in test sensitivities/specificities/accuracy; (iv) identify those factors that significantly increase the risk of infection.

## Methods

### Animals and study period

The study began in July 2019 and ended in September 2020. Thirteen University study centers participated, and each had a recruitment target of 7 cats/month for 12 months, for a total of 84 cats/center. Each cat could only be included in the study once, with no more than 2 cats from the same home/shelter/colony sampled.

### Enrollment and sample collection

Inclusion criteria included: outdoor access; no antiparasitic treatment (endo and/or ecto) in the 3 months prior to enrollment and signed informed consent. Exclusion criteria included: no outdoor access; antiparasitic treatment (endo and/or ecto) in the 3 months prior to enrollment.

Each study center was supplied with: tubes (15 ml) pre-filled with 80% ethanol; flea combs and zip-lock bags; 1 Mini-FLOTAC kit Fill-FLOTAC [14] containing 4 Mini-FLOTAC and 4 Fill-FLOTAC devices (200 tests); instructions for material collection, conservation and analyses.

At enrollment, general information, inclusion criteria, clinical observations, frequency of antiparasitic treatments in the previous 12 months, and eventual signs of ecto-parasitic infection were recorded, and online data collection sheet filled (Supplementary Material Table S1). Any observed ticks/nits (lice) were collected and stored in 80% ethanol pre-filled tubes. Each cat was combed with a flea comb for 5 minutes and collected material was stored in the zip-lock plastic bag at +4°C. Owners were asked to submit at least 7 grams of fresh faeces, which were collected, examined for the eventual presence of proglottids and stored at +4°C.

### Laboratory analyses

All collected material was analyzed at the University laboratories of the 13 study centers.

Material collected with flea combs was examined under a stereomicroscope and the presence of flea/flea debris recorded. Faeces were examined for the presence of proglottids and identified according to [15].

Mini-FLOTAC copromicroscopic examination was carried out on 2 g of faeces in 18 ml of NaCl floating solution (s.g 1200) according to the protocol described in [7]. Minimum/maximum eggs/oocysts/cyst per gram of feces (EPG/OPG/CPG) were calculated. The Baermann test was carried out on 5 g of faeces and examined approximately 12 hours later, according to [16]. Larvae were identified according to [17] and [18].

Study center reference personnel was asked to register with the Castor<sup>®</sup> EDC<sup>®</sup> database [19], managed by the study monitor. Each reference personnel had her/his own login credentials. Results were transcribed into the database, preferably within several days of receipt and analyses.

### Statistical Analyses

Chi-square tests were carried out to evaluate the association between positivity for at least one endo-/ectoparasite infection, *Toxocara cati*, hookworms, *Aelurostrongylus abstrusus*, fleas, and *Otodectes cynotis* and the following categorical variables: sex, geographical area (North, Central, South), life style (exclusive outdoor/predominantly outdoor/predominantly indoor), provenance (privately-owned, shelter, colony), age (< 1 year, 1-5 years, > 5years), antiparasitic treatment in the previous year (yes/no).

Relationships between infection and the variables (“predictors”) was analyzed by multivariable regression analysis applying a stepwise backward elimination methods (IBM SPSS Statistics for Macintosh, Version 27.0.). Statistical significance was set at  $\alpha = 0.05$ .

# Results

## Study population

Severe restrictions on movement/activity were put into place to contain the SARS-CoV-2 pandemic (March-2020 “lockdown”), which resulted in a decrease/lack of cat enrollment on the part of many centers from March 2020 to May 2020 leading to an extension of the study for a further 2 months. A total of 987 cats were enrolled.

Cats were evenly distributed regarding sex. Approximately half the study population (47.3%) was within the age range of 1-5 years, with the remaining evenly distributed between < 1 year (28.1%) and > 5 years of age (24.6%). Over 40% of enrolled cats were from Southern Italy and 69.2% were privately-owned cats. Over 75.8% of the study population lived predominantly outdoor/exclusively outdoor (Table 1).

Most cats, including privately owned animals, had not received anti-parasitic treatment in the year before enrollment. Overall, 539/987 (54.6%) cats from the present study were infected with one or more parasites. Moreover, 13.7% (135/987) had at least two endoparasites and 7.4% (73/987) had at least two arthropod infestations.

## Endoparasites

The overall prevalence of GI and BP nematodes was 35.9% (354/987). *T. cati* was the most prevalent species (253/987; 25.6%), followed by hookworms (98/987; 9.9%). Among BP nematodes, *A. abstrusus* was the most common (76/987; 7.7%), while *Capillaria aerophila* (*Eucoleus aerophilus*; 22/987; 2.2%) and *Troglostrongylus brevior* (12/987; 1.2%) were much less frequently found (Fig. 1 and Table 2). Among GI protozoa, *Cystoisospora felis* was the most common (101/987; 10.2%) while the other species were uncommon (Figure 1 and Table 2). Based on macroscopic examination of proglottids [15], taeniidae were rarely found (*Dipylidium caninum* 3.3%, *Taenia taeniaeformis* 1.3%, *Mesocestoides* spp. 0.1%) (Fig. 1 and Table 2).

Of the 35.9% cats positive for any GI or BP nematodes, 20.1% were female and 15.8% were male. Of the female cats analyzed, 40.0% were positive for endoparasite infection, while of the male cats analyzed, 31.7% showed endoparasites and this difference was statistically significant ( $P=0.007$ ). In addition, when compared to males, females had significantly higher prevalence values for *T. cati* (28.5% vs. 22.8%;  $P=0.04$ ) and hookworms (13.5% vs. 6.3%;  $P<0.001$ ).

Colony cats had significantly higher prevalence of endoparasite infections (45.5%;  $P<0.001$ ) compared to both shelter (31.0%) and privately-owned cats (32.2%). Infection with *A. abstrusus* and hookworms was significantly higher in colony and shelter cats (15.3% and 10.3%, respectively;  $P<0.001$ ) compared to privately-owned cats (4.5%), while prevalence of *T. cati* infection was comparable among the colony/shelter/privately owned cats.

Overall prevalence of endoparasite infection was significantly higher in cats <1 year (49.8%;  $P<0.001$ ) and in cats between 1-5 years of age (36.8%;  $P<0.001$ ), with *T. cati* infection significantly more frequent in cats < 1 year of age (42.6%;  $P<0.001$ ) compared to the other age groups (1-5 years: 24.6%; > 5 years: 8.2%). Infection with hookworms was significantly more frequent in cats between 1-5 years (12.4%;  $P=0.041$ ) compared to the other age categories. Prevalence of *A. abstrusus* was significantly higher in cats < 1 year (9.7%) and between 1-5 years (8.6%;  $P=0.023$ ).

Coccidiosis was more prevalent in younger cats, as is well known (20.6%). Prevalence of endoparasite infection was directly associated with the frequency of outdoor access. Cats with an exclusively outdoor lifestyle had significantly higher infection rates for *A. abstrusus* (14.5%;  $P<0.001$ ) compared to cats living predominantly outdoors or predominantly indoors (5.4% and 2.5%, respectively). Cats with a predominantly/exclusively outdoor lifestyle had significantly higher prevalence values for *T. cati* ( $P=0.02$ ) and hookworms ( $P<0.001$ ). Cats residing in the areas of Southern Italy had significantly higher prevalence values for *T. cati* ( $P<0.001$ ), hookworms ( $P<0.001$ ) and *A. abstrusus* ( $P<0.00$ ).

Monthly prevalence throughout the study was variable (Fig. 2), yet there was no month in which parasites were not observed.

Minimum/maximum EPG/OPG/CPG results for helminths and protozoa were as follows: *T. cati* (from 5 up to 750,000 EPG), *T. leonina* (from 10 up to 950 EPG), hookworms (from 5 up to 8495 EPG), *C. aerophila* (from 5 to 300 EPG), *C. felis* (from 10 up to 21200 OPG), *C. rivolta* (from 5 up to 21200 OPG), and *G. duodenalis* (from 10 up to 550 CPG).

## Ectoparasites

Approximately 35.7% (352/987) of the study population was infested by ectoparasites. The most common were fleas (*C. felis felis*) 29.4% (290/987), followed by ear mites *O. cynotis* 9.8% (97/987). Tick infestation (*Ixodes* spp, *Rhipicephalus* spp.) was uncommon 3.3% (33/987), as was infection by other mites and lice (i.e. *Notoedres* = 5/987, 0.5%; *Trombicula* = 2/987, 0.2%; *Cheyletiella* = 1/987, 0.1%; lice = 8/987, 0.8%) (Fig. 3 and Table 2).

Of the total cat population analysed, prevalence values for ectoparasites were comparable between males (17.3%) and females (18.3%). Of females analysed, 36.6% were infected and of males analysed, 34.8% had ectoparasites. Overall prevalence of flea infestation for the 987 cats recruited was of 14.5% in females vs. 14.9% in males, and for *O. cynotis* 5.7% vs. 4.2%. Considering gender specifically, 28.9% of examined females were infected with fleas and 11.3% with *O. cynotis*, while 29.9% and 8.3% of males were infected, respectively with the two ectoparasites.

Colony cats had significantly higher overall prevalence of ectoparasites (53.5% vs. 31.0% and 28.7%, respectively) ( $P < 0.001$ ), fleas (46.5% vs. 24.1% vs. 22.7%) ( $P < 0.001$ ) and ear mites (17.5% vs. 3.4% vs. 7.8%) ( $P < 0.001$ ), compared to shelter and privately-owned cats. The same was observed for cats with a predominantly/exclusively outdoor lifestyle (35.5% and 51.4% vs. 14.6% indoor lifestyle) ( $P < 0.001$ ).

Overall prevalence for ectoparasite infection was significantly higher in cats <1 year (45.8%) and between 1-5 years (35.5%) ( $P < 0.001$ ) compared to cats > 5 years of age (24.3%). Flea infestation was significantly more prevalent in cats < 1 year (36.5%;  $P < 0.001$ ) and between 1-5 years (29.8%;  $P < 0.001$ ) compared to older cats (20.6%). The same was observed for *O. cynotis*, which showed significantly higher prevalence cats < 1 year of age (12.3%;  $P = 0.02$ ) and between 1-5 years (10.7%;  $P = 0.02$ ) (cats > 5 years 5.3%).

Prevalence of ectoparasites was significantly higher in cats from Central (37.7%) and Southern (42.7%) Italy (compared to North (25.1%) ( $P < 0.001$ ). Interestingly, prevalence of *O. cynotis* infection was significantly higher (19.7%;  $P < 0.001$ ) in cats from Central Italy compared to the North (4.8%) and South (8.5%).

Monthly prevalence throughout the study was variable (Fig. 4). Only 23.8% of the cats had received at least one treatment against endoparasites in the past year, while only 33.6% of the cats had received treatment against ectoparasites in the past year.

### Risk factors for endo-ectoparasites infestation

Significant risk factors as determined from univariate analysis were entered in the multivariable logistic regression model, to address possible confounding factors and to compute adjusted odds ratios. Tables 3 and 4 report results of multivariate analysis, which considered overall prevalence of endoparasite infection, overall prevalence of ectoparasite infection, and prevalence of the most common endoparasites (*T. cati*, hookworms and *A. abstrusus*) and ectoparasites (fleas and *O. cynotis*) as dependent variables and sex, age, provenance, lifestyle, geographical area and anti-parasitic treatment as predictors. Results highlighted that age was predictive for *T. cati* infection, with cats under 1 year of age (OR 7.834, 95% CI 4.609-13.314) and between 1-5 years (OR 3.382, 95% CI 22.021-5.661), but not for hookworms or *A. abstrusus*. Outdoor lifestyle put cats at higher risk for all three nematodes (*T. cati*: OR 2.659, 95% CI 1.622-4.361; hookworms: OR 3.144, 95% CI 1.285-7.691; *A. abstrusus* OR 3.558, 95% CI 1.354-9.354). Significant predictors for ectoparasite infections included living in a colony (OR 1.612, 95% CI 1.114-2.334), exclusive (OR 4.497, 95% CI 2.764-7.318) or predominantly (OR 3.197 95% CI 2.084-4.905) outdoor lifestyle.

## Discussion

The study provides a comprehensive look at the endo- and ectoparasites affecting Italian cats, throughout a 15-month study period. Even though monthly recruitment was brusquely interrupted due to the SARS-CoV-2 pandemic, nearly 1,000 cats were analyzed. Importantly, laboratory analyses were carried out according to standardized protocols which were followed by all centers, thus reducing the risk of variability associated with different test sensitivities/specificities.

The present study also applied univariate and multivariate analyses to evaluate overall risk for endoparasites or ectoparasites and for the most common helminths and arthropods observed.

Results show that feline endo- and ecto-parasites are widespread in Italy, with varying prevalence across the different regions. Approximately 55.0% (539/987) of cats from the present study were infected with at least one internal or external parasite. This compares well with results from [1] that evaluated parasite infection of the European owned cat population, demonstrating that more than half (50.7%) of the cats studied were infected with one or more endo- and/or ectoparasites.

Among gastro-intestinal helminths, *T. cati* was the most frequently found (25.6% of enrolled cats). This is higher than that reported by a previous Italian multicenter study [4], which highlighted a prevalence of 21.6%, and also higher than the mean European prevalence value reported in 2017 by [2] (14.5%). The prevalence is, however, in line with other surveys conducted in Italy [5, 6]. In the present study, risk for *T. cati* infection included age (< 5 years of age), an exclusively outdoor lifestyle and living in Southern Italy. Pre- and perinatal transmission and age immunity are well-known for *Toxocara* spp., while outdoor access favors exposure to both highly resistant eggs contaminating the environment and to paratenic hosts [20]. Warmer climatic conditions in Southern Italy and islands likely seems to increase the presence and persistence of infective stages of the parasite.

Surprisingly, hookworms were the second most frequent group of nematodes diagnosed in the study population (9.9%). Giannelli et al. [2] reported a mean prevalence of 4.5%, while [4] reported a prevalence of 4.9%. In the present study, hookworm infection was associated with gender (females were at increased risk), predominantly outdoor access, living in Southern Italy and lack of anthelmintic treatment. Age was not a significant risk predictor. Indeed, it is assumed that there is no transmammary transmission of hookworms from the queen to her kittens [21]

As mentioned above, warmer climatic conditions in Southern Italy may increase the presence and persistence of infective stages of hookworms. For transmission to occur, hookworms need to develop into infective larvae in the soil from eggs passed in the host's stool and higher temperature and humidity (tropical and subtropical climates) provide an adequate environment for this growth stage [22].

The feline lungworm *A. abstrusus* was present in 7.7% of analysed cats. Mean prevalence in Europe according to [2] was 8.2%, while recent data from Italy [4] reported a prevalence of 10.3%. Multivariate analysis indicated exclusively outdoor lifestyle, living in Southern Italy and lack of anthelmintic treatment as significant risk factors for *A. abstrusus*. Outdoor access has been reported previously as an important risk factor for *A. abstrusus* [23]. While the geographical distribution of feline lungworms tends to be patchy but stable in endemic hotspots [2], interpretation of geographical location as a risk factor should be done with caution and any geographic location reporting autochthonous circulation of the parasite should be considered potentially endemic. Traversa et al. [4] reported a 20.0% prevalence of *A. abstrusus* in Piedmont, while in the present study only 1.2% of cats were infected. Giannelli et al. [2] reported that 16.7% of cats from the province of Bari were infected with *A. abstrusus*, while in the present study the prevalence was 2.4%. Values from provinces of Sassari (SS) and Messina (ME), on the other hand, were higher in the present study (20.9% and 22.4%, respectively) (Table 2), compared to data reported by [2] (11.6% and 15.3%, respectively), but lower than that reported by [24] in a previous study in Sardinia. Interestingly, univariate analysis showed cats > 5 years of age had significantly higher prevalence for *A. abstrusus*, but age was not confirmed as a risk factor following multivariate evaluation. As expected, larvae of *T. brevior* were found mostly in cats from Southern regions. Nevertheless, it is worth mentioning that, in the present study, this potentially fatal metastrongyloid was also found in two cats in Northern Italy, indicating an apparent northward geographical expansion.

Mini-FLOTAC in combination with Fill-FLOTAC was shown to be user-friendly and safe, with a wide diagnostic range. These features are particularly useful for monitoring and control programs in which large numbers of fecal samples need to be processed rapidly and safely. The harmonized use of the Mini-FLOTAC technique allowed the qualitative and quantitative analysis of parasite load without the need for specialized equipment.

Fleas were the most common ectoparasite found in the present study (29.4%). Cooper et al. [25] reported similar prevalence values in a recent nationwide study in the United Kingdom. Beugnet et al. [1] reported prevalence values ranging from 3.6% in Bari to 31.4% in Naples. None of the study centers in the present study reported values less than 14.0%. Multivariate analysis showed that infestation was associated with young age (< 1 year), living in a colony, predominantly/exclusively outdoor lifestyle, living in Central and Southern Italy and lack of ectoparasitic treatment. Cooper et al. [25] have reported geographical differences (prevalence declining from South to North in UK) and no treatment as significant predictors of risk for flea infestation. Beugnet et al. [1] identified outdoor access as the only risk factor in multivariate analysis.

The ear mite *O. cynotis* was present in 9.8% of enrolled cats. Beugnet et al. [1] reported prevalence values of 40.3% in cats from Bari and 21.8% in cats from the area of Naples. In Tuscany (central Italy), *O. cynotis* was identified in 66.1% cats with otitis externa [26]. Age < 1 year, living in a colony, having a predominantly outdoor lifestyle and coming from Central Italy were all factors identified as increasing risk of ear mite infection in cats. *O. cynotis* is transmitted by direct contact and is highly contagious. Young cats are more playful and likely have more direct contact with other cats.

The main limitation of the present study is the potential effect of bias, based on inclusion criteria. Indeed, similar to a previous multicenter study [1], here only cats that had not received antiparasitic treatment in the previous three months were enrolled. Furthermore, outdoor access was necessary for enrolment.

## Conclusions

The results of this study highlight the fact that both ecto- and endoparasites are still common in cats throughout Italy. Interestingly, of the 239 cats with a predominantly indoor lifestyle, 31.8% were affected by endo-ectoparasites, suggesting that parasiticide treatment is more important than lifestyle. Therefore, given the zoonotic consideration and the clinical importance, it is strongly advisable to promote effective and regular parasite control in cats, with adequate frequencies of treatment for both internal and external parasites.

It is interesting to note that there was no month in which endo-ectoparasites could not be found, suggesting that cats can be infected all year round. This would imply that parasite infection should not be considered seasonal, but that control should be year-round. However, only 23.8% of the cats had received at least one treatment against endoparasites in the past year, while only 33.6% of the cats had received treatment against ectoparasites in the past year.

The European Council for the Control of Companion Animal Parasites (ESCCAP) recommends “year-round, life-long” parasite control. Many privately-owned cats spend a significant amount of time outdoors and are exposed to parasites. Practitioners need to inform their clients of the risks and recommend periodic anti-parasitic treatment.

Interestingly, Southern Italy continues to show higher prevalence of parasite infection in cats. This may be due to climatic, social or economic factors and practitioners working in these areas should be particularly attentive.

Finally, zoonotic parasites and vectors of human disease are still widespread in cats, confirming the need to protect the human-animal bond and the application of the One-Health concept.

## Abbreviations

GI: gastro-intestinal nematodes

BP: bronco-pulmonary

## Declarations

### ***Ethics approval and consent to participate***

The cats' owners signed an informed consent authorizing the use of faeces and hair for the study.

### ***Consent for publication***

Informed consent was obtained from all individual participants included in the study.

### ***Availability of data and material***

All data generated or analysed during this study are included in this published article

### ***Competing interests***

The authors declare that they have no competing interests

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Experiments were conceived and designed by all authors. Sampling was done by all authors. Experiments were performed by all authors. The data were analyzed by MG and FSB. Statistical analyses were performed by FSB. The manuscript was written by LK, MG and AV and critically revised by all authors. All authors read and approved the final manuscript.

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## Tables

Table 1. Features of studied population (counts and percentages)

|                          |                             |                             |                            |
|--------------------------|-----------------------------|-----------------------------|----------------------------|
| <b>Sex</b>               | <b>Male</b>                 | <b>Female</b>               |                            |
|                          | 492 (49.8%)                 | 495 (50.2)                  |                            |
| <b>Status</b>            | <b>Non sterilized</b>       | <b>Sterilized</b>           |                            |
|                          | 438 (44.4%)                 | 549 (55.6%)                 |                            |
| <b>Provenance</b>        | <b>Shelter</b>              | <b>Colony</b>               | <b>Privately-owned</b>     |
|                          | 29 (2.9%)                   | 275 (27.9%)                 | 683 (69.2%)                |
| <b>Lifestyle</b>         | <b>Predominantly indoor</b> | <b>Predominatly outdoor</b> | <b>Exclusively outdoor</b> |
|                          | 239 (24.2%)                 | 423 (42.9%)                 | 325 (32.9%)                |
| <b>Geographical area</b> | <b>North</b>                | <b>Central</b>              | <b>South</b>               |
|                          | 331 (33.5%)                 | 223 (22.6%)                 | 433 (43.9%)                |
| <b>Age</b>               | <b>&lt; 1 y</b>             | <b>1-5 y</b>                | <b>&gt; 5 y</b>            |
|                          | 277 (28.1%)                 | 467 (47.3%)                 | 243 (24.6%)                |

Table 2 Prevalence (%), positive cats, of endo-ectoparasites reported from the 13 Study Centers in Italy.

TO: Tourin, MI: Milan, PD: Padua, PR: Parma, BO: Bologna, PI: Pisa, PG: Perugia, TE: Teramo, BA: Bari, CZ: Catanzaro, NA: Naples, SS: Sassari, ME: Messina

| <b>Gastrointestinal parasites</b> |              |           |           |           |                |           |           |           |              |           |           |           |           |             |
|-----------------------------------|--------------|-----------|-----------|-----------|----------------|-----------|-----------|-----------|--------------|-----------|-----------|-----------|-----------|-------------|
|                                   | <b>North</b> |           |           |           | <b>Central</b> |           |           |           | <b>South</b> |           |           |           |           |             |
| <b>Study Centers</b>              | <b>TO</b>    | <b>MI</b> | <b>PD</b> | <b>PR</b> | <b>BO</b>      | <b>PI</b> | <b>PG</b> | <b>TE</b> | <b>BA</b>    | <b>CZ</b> | <b>NA</b> | <b>SS</b> | <b>ME</b> | <b>TOT</b>  |
| N. cats                           | 85           | 84        | 64        | 84        | 14             | 49        | 89        | 85        | 84           | 84        | 89        | 91        | 85        | <b>987</b>  |
| <i>Toxocara cati</i>              | 8            | 21        | 19        | 12        | 8              | 14        | 11        | 16        | 15           | 37        | 29        | 28        | 35        | <b>253</b>  |
| Prevalence                        | 9.4          | 25.0      | 29.7      | 14.3      | 57.1           | 28.6      | 12.4      | 18.8      | 17.9         | 44.0      | 32.6      | 30.8      | 41.2      | <b>25.6</b> |
| <i>Toxascaris leonina</i>         | 4            | 0         | 0         | 1         | 0              | 0         | 0         | 0         | 1            | 2         | 0         | 1         | 0         | <b>9</b>    |
| Prevalence                        | 4.7          | 0.0       | 0.0       | 1.2       | 0.0            | 0.0       | 0.0       | 0.0       | 1.2          | 2.4       | 0.0       | 1.1       | 0.0       | <b>0.9</b>  |
| <b>Hookworms</b>                  | 1            | 2         | 5         | 12        | 0              | 0         | 1         | 6         | 4            | 12        | 9         | 32        | 14        | <b>98</b>   |
| Prevalence                        | 1.2          | 2.4       | 7.8       | 14.3      | 0.0            | 0.0       | 1.1       | 7.1       | 4.8          | 14.3      | 10.1      | 35.2      | 16.5      | <b>9.9</b>  |
| <i>Spirocerca lupi</i>            | 0            | 0         | 0         | 0         | 0              | 0         | 0         | 0         | 0            | 0         | 0         | 0         | 0         | <b>0</b>    |
| Prevalence                        | 0.0          | 0.0       | 0.0       | 0.0       | 0.0            | 0.0       | 0.0       | 0.0       | 0.0          | 0.0       | 0.0       | 0.0       | 0.0       | <b>0.0</b>  |
| <i>Physaloptera spp.</i>          | 0            | 0         | 0         | 0         | 0              | 0         | 0         | 0         | 0            | 0         | 0         | 0         | 0         | <b>0</b>    |
| Prevalence                        | 0.0          | 0.0       | 0.0       | 0.0       | 0.0            | 0.0       | 0.0       | 0.0       | 0.0          | 0.0       | 0.0       | 0.0       | 0.0       | <b>0.0</b>  |
| <i>Strongyloides stercoralis</i>  | 0            | 0         | 0         | 0         | 0              | 1         | 0         | 0         | 0            | 0         | 0         | 0         | 0         | <b>1</b>    |
| Prevalence                        | 0.0          | 0.0       | 0.0       | 0.0       | 0.0            | 2.0       | 0.0       | 0.0       | 0.0          | 0.0       | 0.0       | 0.0       | 0.0       | <b>0.1</b>  |
| <i>Cystoisospora felis</i>        | 6            | 4         | 0         | 5         | 0              | 2         | 7         | 9         | 6            | 13        | 17        | 23        | 9         | <b>101</b>  |
| Prevalence                        | 7.1          | 4.8       | 0.0       | 6.0       | 0.0            | 4.1       | 7.9       | 10.6      | 7.1          | 15.5      | 19.1      | 25.3      | 10.6      | <b>10.2</b> |
| <i>Cystoisospora rivolta</i>      | 1            | 0         | 0         | 0         | 0              | 0         | 1         | 2         | 0            | 0         | 8         | 2         | 0         | <b>14</b>   |
| Prevalence                        | 1.2          | 0.0       | 0.0       | 0.0       | 0.0            | 0.0       | 1.1       | 2.4       | 0.0          | 0.0       | 9.0       | 2.2       | 0.0       | <b>1.4</b>  |
| <i>Giardia duodenalis</i>         | 0            | 0         | 0         | 2         | 0              | 0         | 2         | 0         | 0            | 0         | 3         | 4         | 0         | <b>11</b>   |
| Prevalence                        | 0.0          | 0.0       | 0.0       | 2.4       | 0.0            | 0.0       | 2.2       | 0.0       | 0.0          | 0.0       | 3.4       | 4.4       | 0.0       | <b>1.1</b>  |
| <i>Dipylidium caninum</i>         | 0            | 0         | 0         | 1         | 2              | 1         | 8         | 1         | 0            | 13        | 2         | 0         | 5         | <b>33</b>   |
| Prevalence                        | 0.0          | 0.0       | 0.0       | 1.2       | 14.3           | 2.0       | 9.0       | 1.2       | 0.0          | 15.5      | 2.2       | 0.0       | 5.9       | <b>3.3</b>  |
| <i>Taenia taeniformis</i>         | 0            | 0         | 1         | 2         | 0              | 0         | 3         | 0         | 5            | 0         | 0         | 0         | 2         | <b>13</b>   |
| Prevalence                        | 0.0          | 0.0       | 1.6       | 2.4       | 0.0            | 0.0       | 3.4       | 0.0       | 6.0          | 0.0       | 0.0       | 0.0       | 2.4       | <b>1.3</b>  |
| <i>Mesocestoides spp.</i>         | 0            | 0         | 0         | 0         | 0              | 0         | 0         | 0         | 0            | 0         | 1         | 0         | 0         | <b>1</b>    |
| Prevalence                        | 0.0          | 0.0       | 0.0       | 0.0       | 0.0            | 0.0       | 0.0       | 0.0       | 0.0          | 0.0       | 1.1       | 0.0       | 0.0       | <b>0.1</b>  |

| <b>Lung parasites</b>             |              |           |           |           |                |           |           |           |              |           |           |           |           |            |
|-----------------------------------|--------------|-----------|-----------|-----------|----------------|-----------|-----------|-----------|--------------|-----------|-----------|-----------|-----------|------------|
|                                   | <b>North</b> |           |           |           | <b>Central</b> |           |           |           | <b>South</b> |           |           |           |           |            |
| <b>Study Centers</b>              | <b>TO</b>    | <b>MI</b> | <b>PD</b> | <b>PR</b> | <b>BO</b>      | <b>PI</b> | <b>PG</b> | <b>TE</b> | <b>BA</b>    | <b>CZ</b> | <b>NA</b> | <b>SS</b> | <b>ME</b> | <b>TOT</b> |
| N. cats                           | 85           | 84        | 64        | 84        | 14             | 49        | 89        | 85        | 84           | 84        | 89        | 91        | 85        | <b>987</b> |
| <i>Aelurostrongylus abstrusus</i> | 1            | 4         | 3         | 0         | 0              | 0         | 5         | 6         | 2            | 1         | 16        | 19        | 19        | <b>76</b>  |
| Prevalence                        | 1.2          | 4.8       | 4.7       | 0.0       | 0.0            | 0.0       | 5.6       | 7.1       | 2.4          | 1.2       | 18.0      | 20.9      | 22.4      | <b>7.7</b> |
| <i>Troglostrongylus brevior</i>   | 0            | 0         | 0         | 0         | 2              | 0         | 0         | 3         | 1            | 0         | 4         | 2         | 0         | <b>12</b>  |
| Prevalence                        | 0.0          | 0.0       | 0.0       | 0.0       | 14.3           | 0.0       | 0.0       | 3.5       | 1.2          | 0.0       | 4.5       | 2.2       | 0.0       | <b>1.2</b> |
| <i>Capillaria aerophila</i>       | 1            | 3         | 3         | 2         | 0              | 0         | 1         | 7         | 1            | 0         | 1         | 2         | 1         | <b>22</b>  |
| Prevalence                        | 1.2          | 3.6       | 4.7       | 2.4       | 0.0            | 0.0       | 1.1       | 8.2       | 1.2          | 0.0       | 1.1       | 2.2       | 1.2       | <b>2.2</b> |

| <b>Ectoparasites</b>         |              |           |           |           |                |           |           |           |              |           |           |           |           |             |
|------------------------------|--------------|-----------|-----------|-----------|----------------|-----------|-----------|-----------|--------------|-----------|-----------|-----------|-----------|-------------|
|                              | <b>North</b> |           |           |           | <b>Central</b> |           |           |           | <b>South</b> |           |           |           |           |             |
| <b>Study Centers</b>         | <b>TO</b>    | <b>MI</b> | <b>PD</b> | <b>PR</b> | <b>BO</b>      | <b>PI</b> | <b>PG</b> | <b>TE</b> | <b>BA</b>    | <b>CZ</b> | <b>NA</b> | <b>SS</b> | <b>ME</b> | <b>TOT</b>  |
| N. cats                      | 85           | 84        | 64        | 84        | 14             | 49        | 89        | 85        | 84           | 84        | 89        | 91        | 85        | <b>987</b>  |
| <b>Flea</b>                  | 16           | 12        | 17        | 14        | 6              | 25        | 23        | 13        | 32           | 15        | 28        | 45        | 44        | <b>290</b>  |
| Prevalence                   | 18.8         | 14.3      | 26.6      | 16.7      | 42.9           | 51.0      | 25.8      | 15.3      | 38.1         | 17.9      | 31.5      | 49.5      | 51.8      | <b>29.4</b> |
| <b>Ticks</b>                 | 2            | 2         | 2         | 3         | 3              | 10        | 1         | 2         | 1            | 2         | 0         | 5         | 0         | <b>33</b>   |
| Prevalence                   | 2.4          | 2.4       | 3.1       | 3.6       | 21.4           | 20.4      | 1.1       | 2.4       | 1.2          | 2.4       | 0.0       | 5.5       | 0.0       | <b>3.3</b>  |
| <b>Lices</b>                 | 0            | 0         | 2         | 2         | 0              | 0         | 1         | 0         | 1            | 1         | 0         | 1         | 0         | <b>8</b>    |
| Prevalence                   | 0.0          | 0.0       | 3.1       | 2.4       | 0.0            | 0.0       | 1.1       | 0.0       | 1.2          | 1.2       | 0.0       | 1.1       | 0.0       | <b>0.8</b>  |
| <i>Otodectes cynotis</i>     | 3            | 1         | 6         | 5         | 1              | 7         | 34        | 3         | 14           | 2         | 14        | 3         | 4         | <b>97</b>   |
| Prevalence                   | 3.5          | 1.2       | 9.4       | 6.0       | 7.1            | 14.3      | 38.2      | 3.5       | 16.7         | 2.4       | 15.7      | 3.3       | 4.7       | <b>9.8</b>  |
| <i>Notoedres cati</i>        | 0            | 0         | 0         | 0         | 0              | 0         | 1         | 0         | 0            | 1         | 2         | 0         | 1         | <b>5</b>    |
| Prevalence                   | 0.0          | 0.0       | 0.0       | 0.0       | 0.0            | 0.0       | 1.1       | 0.0       | 0.0          | 1.2       | 2.2       | 0.0       | 1.2       | <b>0.5</b>  |
| <i>Trombicula autumnalis</i> | 0            | 0         | 0         | 0         | 0              | 1         | 0         | 0         | 0            | 0         | 1         | 0         | 0         | <b>2</b>    |
| Prevalence                   | 0.0          | 0.0       | 0.0       | 0.0       | 0.0            | 2.0       | 0.0       | 0.0       | 0.0          | 0.0       | 1.1       | 0.0       | 0.0       | <b>0.2</b>  |
| <i>Cheyletiella</i> spp.     | 0            | 0         | 0         | 0         | 0              | 0         | 0         | 0         | 0            | 0         | 0         | 1         | 0         | <b>1</b>    |
| Prevalence                   | 0.0          | 0.0       | 0.0       | 0.0       | 0.0            | 0.0       | 0.0       | 0.0       | 0.0          | 0.0       | 0.0       | 1.1       | 0.0       | <b>0.1</b>  |
| <i>Demodex</i> spp.          | 0            | 0         | 0         | 0         | 0              | 0         | 0         | 0         | 0            | 0         | 0         | 0         | 0         | <b>0</b>    |
| Prevalence                   | 0.0          | 0.0       | 0.0       | 0.0       | 0.0            | 0.0       | 0.0       | 0.0       | 0.0          | 0.0       | 0.0       | 0.0       | 0.0       | <b>0.0</b>  |
| <i>Thelazia</i> spp.         | 0            | 0         | 0         | 0         | 0              | 1         | 1         | 0         | 0            | 1         | 1         | 0         | 0         | <b>4</b>    |
| Prevalence                   | 0.0          | 0.0       | 0.0       | 0.0       | 0.0            | 2.0       | 1.1       | 0.0       | 0.0          | 1.2       | 1.1       | 0.0       | 0.0       | <b>0.4</b>  |

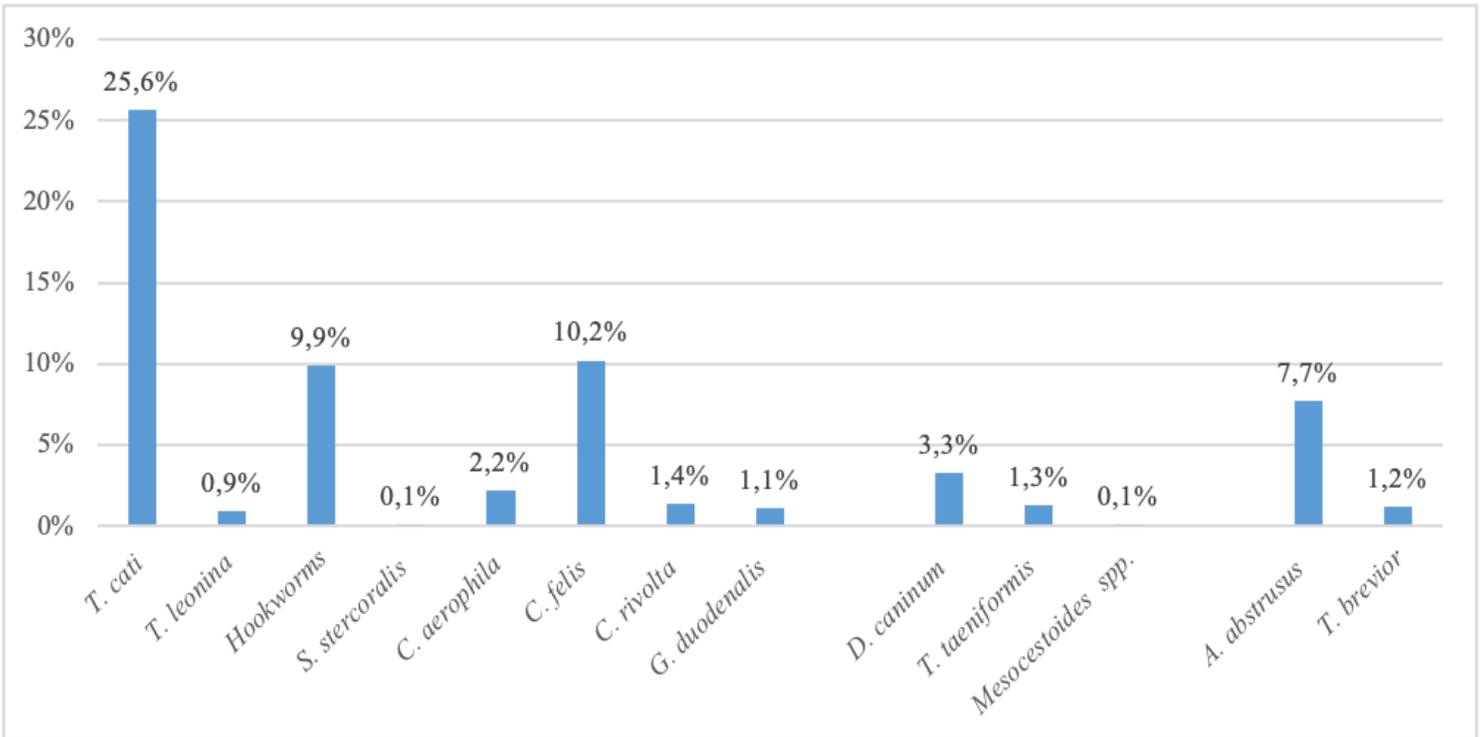
Table 3. Multivariable regression analyses for Endoparasites. (IBM SPSS Statistics for Macintosh, Version 27.0.). Statistical significance was set at  $\alpha = 0.05$ .

| <b>1. Endoparasite infection</b>            |                |                       |
|---|----------------|-----------------------|
| <b>Significant Predictor of risk</b>        | <b>P value</b> | <b>OR (95% CI)</b>    |
| Female                                      | 0.047          | 1.331 (1.003 - 1.765) |
| Age (<1Y)                                   | 0.000          | 3.953 (2.587 - 6.041) |
| Age (1-5Y)                                  | 0.000          | 2.198 (1.483- 3.258)  |
| Exclusively outdoor                         | 0.000          | 3.515 (2.223- 5.560)  |
| South                                       | 0.000          | 2.052 (1.476- 2.853)  |
| No treatment                                | 0.018          | 1.517 (1.074- 2.143)  |
| <b>2. <i>Toxocara cati</i></b>              |                |                       |
| <b>Significant Predictor of risk</b>        | <b>P value</b> | <b>OR (95% CI)</b>    |
| Age (<1Y)                                   | 0.000          | 7.834 (4.609-13.314)  |
| Age (1-5Y)                                  | 0.000          | 3.382 (2.021-5.661)   |
| Exclusively outdoor                         | 0.000          | 2.659 (1.622-4.361)   |
| South                                       | 0.018          | 1.542 (1.076-2.208)   |
| <b>3. Hookworms</b>                         |                |                       |
| <b>Significant Predictor of risk</b>        | <b>P value</b> | <b>OR (95% CI)</b>    |
| Female                                      | 0.001          | 2.218 (1.390-3.537)   |
| Predominantly outdoor                       | 0.012          | 3.144 (1.285-7.691)   |
| South                                       | 0.000          | 3.277 (1.874-5.730)   |
| No treatment                                | 0.006          | 2.472 (1.300-4.700)   |
| <b>4. <i>Aelurostrongylus abstrusus</i></b> |                |                       |
| <b>Significant Predictor of Risk</b>        | <b>P value</b> | <b>OR (95% CI)</b>    |
| Exclusively outdoor                         | 0.010          | 3.558 (1.354-9.354)   |
| South                                       | 0.000          | 5.480 (2.498-12.024)  |
| No treatment                                | 0.020          | 2.440 (1.154- 5.160)  |

Table 4 Multivariable analyses for Ectoparasites.

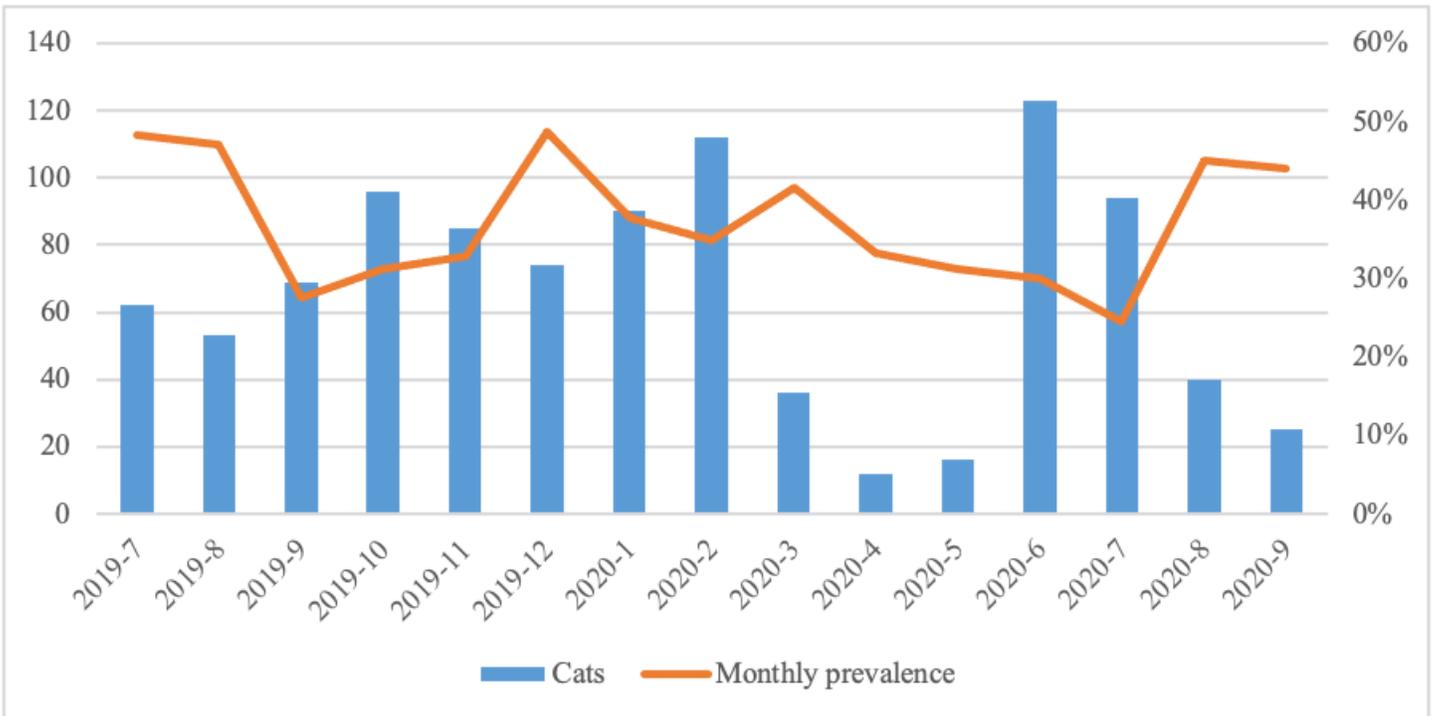
| <b>1. Ectoparasite infection</b>     |                |                       |
|--------------------------------------|----------------|-----------------------|
| <b>Significant Predictor of risk</b> | <b>P value</b> | <b>OR (95% CI)</b>    |
| Age (<1Y)                            | 0.000          | 2.290 (1.518-3.455)   |
| Colony                               | 0.011          | 1.612 (1.114-2.334)   |
| Predominantly outdoor                | 0.000          | 3.197 (2.084 -4.905)  |
| Exclusively outdoor                  | 0.000          | 4.497 (2.764 -7.318)  |
| Central                              | 0.001          | 1.962 (1.320 - 2.916) |
| South                                | 0.001          | 1.757 (1.256-2.458)   |
| <b>2. Fleas</b>                      |                |                       |
| <b>Significant Predictor of risk</b> | <b>P value</b> | <b>OR (95% CI)</b>    |
| Age (<1Y)                            | 0.017          | 1.699 (1.099-2.626)   |
| Colony                               | 0.049          | 1.459 (1.001-2.125)   |
| Predominantly outdoor                | 0.000          | 3.294 (2.024 -5.361)  |
| Exclusively outdoor                  | 0.000          | 5.092 (2.974-8.718)   |
| Central                              | 0.030          | 1.606 (1.046 -2.465)  |
| South                                | 0.000          | 2.069 (1.448 – 2.958) |
| No treatment                         | 0.002          | 1.725 (1.215-2.449)   |
| <b>3. <i>Otodectes cynotis</i></b>   |                |                       |
| <b>Significant Predictor of risk</b> | <b>P value</b> | <b>OR (95% CI)</b>    |
| Age (< 1y)                           | 0.017          | 2.377 (1.164 – 4.854) |
| Colony                               | 0.000          | 3.232 (1.801 – 5.803) |
| Predominantly outdoor                | 0.030          | 2.193 (1.077-4.462)   |
| Central                              | 0.000          | 4.526 (2.652 – 7.724) |

## Figures



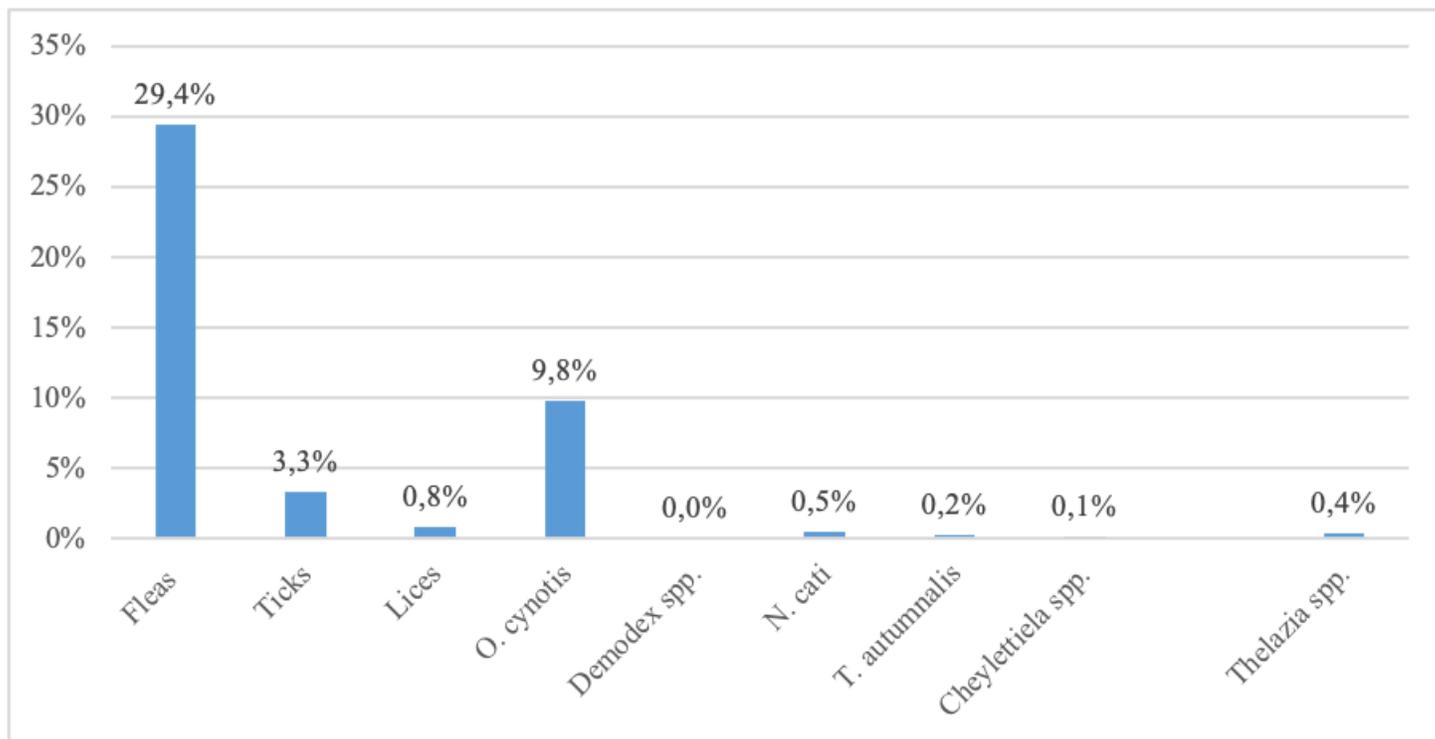
**Figure 1**

Overall prevalence of gastro-intestinal (GI) and bronco-pulmonary (BP) endoparasites.



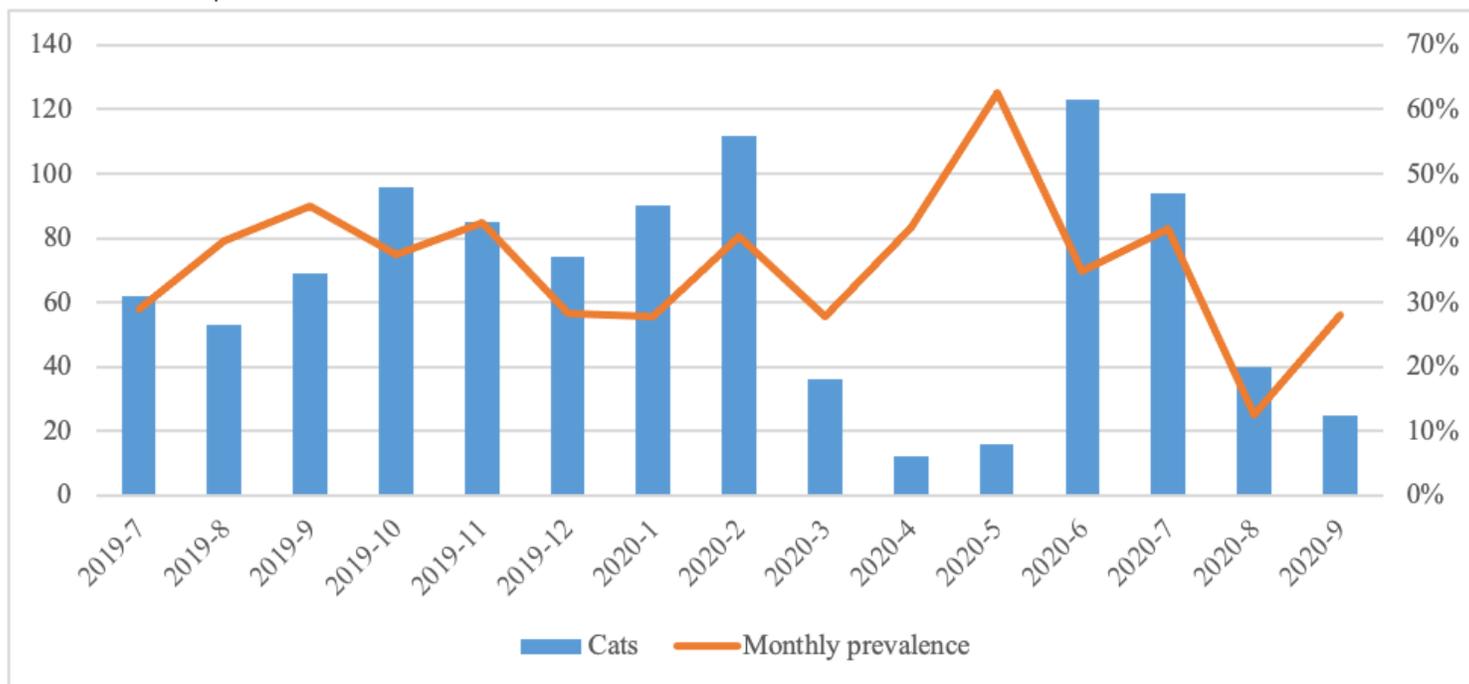
**Figure 2**

Endoparasite prevalence according to monthly evaluation.



**Figure 3**

Prevalence of ectoparasite infection.



**Figure 4**

Monthly prevalence of ectoparasite infections.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [graphicalabstract.png](#)
- [App.1Enrolment1.docx](#)