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SHORT COMMUNICATION

Occurrence and zoonotic potential of endoparasites in cats of Cyprus and a new distribution area for *Troglostrongylus brevior*

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Abstract The present study investigated for the first time the occurrence of pulmonary and intestinal parasites of cats in Cyprus. Cats from five districts of Cyprus (Lefkosia, Lemesos, Larnaka, Pafos and Ammochostos) were examined by classical parasitological methods and the identity of lungworm larvae, whenever present, was confirmed by PCR-coupled sequencing. A total of 185 cats, 48 living exclusively indoors and 137 with outdoor access, were included in the study. Parasites were found in 66 (35.7%) of the examined cats, i.e. *Toxocara cati* (12%), *Cystoisospora rivolta* (12%), *Joyeuxiella/Diplopylidium* spp. (7%), *Giardia* spp. (6.5%), *Troglostrongylus brevior* (5%), *Cystoisospora felis* (2.5%), *Aelurostrongylus abstrusus* (2%), *Taenia* spp. (0.5%) and *Dipylidium caninum* (0.5%). Mixed infections were recorded in 18 cats. Cats that lived exclusively indoors or had received an antiparasitic treatment in the last 6 months were less likely to be infected ($p < 0.05$). Moreover, cats younger than 1 year old were more likely to shed first-stage larvae of *T. brevior* ($p = 0.04$). The present study shows that cats in Cyprus are infected at a high percentage by a variety of parasites that potentially affect their health and also, in some cases (i.e. *T. cati*, *D. caninum*, *Giardia* spp.), may have an impact on human health. Moreover, it was revealed that *T. brevior*, a lungworm of emerging significance, is present on the island, rendering Cyprus the easternmost distribution border of this parasite in Europe to date.

Keywords Cat · Cyprus · Endoparasites · *Troglostrongylus brevior* · Zoonotic

Introduction

The domestic cat (*Felis silvestris catus*) is one of the most popular pets all over the globe. The number of pet cats in the European Union is currently estimated at over 74 million, but it is most likely underrated. In most communities, cats are kept indoors, outdoors or both, as companion animals, contributors to pest control and recently, in animal-assisted interventions (Cherniack and Cherniack 2014; Kedanis 2016). Endoparasites are among the major concerns regarding the health and welfare of cats. In particular, protists and helminths, principally inhabiting the intestinal and respiratory tracts, are commonly found [for example, in 35% of a cat population studied in a recent survey in Europe (Giannelli et al. 2017)] and, in various cases, are agents of severe diseases in their hosts (Traversa 2012; Beugnet et al. 2014). Moreover, some of these parasites have zoonotic potential, as they can also infect humans, causing diseases that, under particular circumstances, can be serious (Traversa 2012; Baneth et al. 2016). In recent years, a number of surveys have investigated feline endoparasites in Europe and other continents, revealing high prevalence of infection and the presence of parasites that may also be involved in human diseases (Abu-Madi et al. 2007; Ramos et al. 2013; Beugnet et al. 2014; Di Cesare et al. 2015a; Hoopes et al. 2015; Little et al. 2015; Rodriguez-Ponce et al. 2016; Diakou et al. 2017;

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Giannelli et al. 2017; Kostopoulou et al. 2017; Szwabe and Blaszkowska 2017).

Although the parasites of domestic animals appear to have been meticulously researched and their epizootiology and pathogenesis have been certified to a great extent, newly discovered parasite-host relationships remain of acute scientific interest. One key example is *Troglostrongylus brevior*, a respiratory nematode that was, until recently, considered a parasite affecting almost exclusively wild felids (Traversa and Di Cesare 2013; Brianti et al. 2014). Nevertheless, in recent years, it has been found in domestic cats in Southern Europe, with a noteworthy prevalence in some regions (Brianti et al. 2014; Di Cesare et al. 2015a; Diakou et al. 2015).

Despite the many intensive investigations of feline parasites conducted throughout the world, there are still many areas, even in the so-called western or developed world, for which no relevant information has ever been reported. The Republic of Cyprus in the Eastern Mediterranean is an international hub of tourism, business and services and one of the fastest growing EU economies (population 1,253,655, density 123.4/km^2, gross domestic product per capita \$23,352, estimated for 2016, according to the National Statistical Service). Although this island has a large number of cats living as owned pets, free-roaming or stray animals, no surveys on the parasites of the intestinal and respiratory tract of cats in Cyprus have been conducted thus far. In order to fill in this gap of information and contribute to our knowledge about the epizootiology and distribution of feline parasites, the present survey investigated, for the first time, the occurrence of pulmonary and intestinal parasites of cats in Cyprus.

Materials and methods

Individual faecal samples of 185 cats living in areas under the control of the Republic of Cyprus, in the districts of Lefkosia, Lemesos, Larnaka, Pafos and Ammochostos, were collected with the permission and collaboration of their owners or the personnel of the shelters that hosted them. Data on sex, age, district, lifestyle (indoors vs outdoors) and timing of the last anthelminthic treatment were recorded for all animals. Faecal samples were stored at 4 °C until examined, no later than 7 days after their collection.

Faecal examinations included $ZnSO^4$ flotation, merthiolate iodine formaldehyde (MIF)-ether sedimentation and Baermann method (MAAF 1986; Thienpont et al. 1986). Identification of parasitic stages (eggs, larvae, cysts and oocysts) was based on their morphologic and morphometric features (Taylor et al. 2007; Brianti et al. 2014).

Species identification of lungworm first-stage larvae (L1) collected by Baermann technique was confirmed by a multiplex semi-nested PCR for the simultaneous detection of *Aelurostrongylus abstrusus* and *T. brevior* specific DNA (Di Cesare et al. 2015c). All samples that scored PCR positive were then subjected to a PCR-coupled sequencing analysis of a region within the gene encoding the mitochondrial cytochrome c oxidase subunit 1 (Traversa et al. 2017).

Results were statistically analysed with the Chi-square test of independence and the Fisher's exact test ($p < 0.05$). Moreover, a cross-tabulation table of association for two variables (district and infection) with one control variable (lifestyle) was calculated. The software used for the statistical analysis was IBM SPSS Statistics 23, 24.

Results

Forty-eight of the 185 cats examined lived exclusively indoors while 137 lived outdoors or had outdoor access. Parasites were found in 66 (35.7%) of the cats and, more precisely, in 5 of the 48 (10.4%) cats living indoors and in 61 of the 137 (44.5%) cats with outdoor access. The parasites found in total (in order of prevalence) were *Toxocara cati* (12%), *Cystoisospora rivolta* (12%), *Joyeuxiella/Diplopylidium* spp. (7%), *Giardia* spp. (6.5%), *Troglostrongylus brevior* (5%), *Cystoisospora felis* (2.5%), *Aelurostrongylus abstrusus* (2%), *Dipylidium caninum* (0.5%) and *Taenia* spp. (0.5%). Mixed infections were recorded in 18 (9.7%) cats (Table 1). The prevalence of infection in the different districts was 28.7% in Lemesos ($n = 87$), 50% in Pafos ($n = 64$), 15.8% in Lefkosia ($n = 19$), 45.6% in Larnaka ($n = 11$) and 25% in Ammochostos ($n = 4$). Details on prevalence of each parasite are presented in Table 1. Some of the cats showed mild clinical signs such as diarrhoea, coughing or moderate body condition, but these were not always associated with parasitic infection.

The statistical analysis showed that cats with outdoor lifestyle were more likely to be infected than cats that lived exclusively indoors ($p < 0.0005$), with a ratio of infection prevalence of 32.4/3.2 (Table 1). Similarly, cats that had received a deworming treatment within the last 6 months were less likely to be infected ($p = 0.01$). Significant association ($p = 0.04$) was also found between age (< 1 year) and the presence of *T. brevior* larvae in the faeces of the cats. Finally, the cross-tabulation table of association showed that, of the 5 districts, Lefkosia had statistically significant ($p < 0.05$) lower prevalence of infection, while Pafos had the highest prevalence of infection ($p < 0.05$), regardless of the lifestyle of the animals.

Morphological identification of lungworm L1 was in all cases in accordance with the molecular identification. The sequencing of all samples identified as *T. brevior* revealed that they belonged to the haplotype II, recently characterised from domestic and wildcats from Italy and from domestic cats on the island of Mykonos, Greece (Traversa et al. 2017).

Table 1 Prevalence (% ± 95% confidence interval, CI) of parasites found by faecal examinations in cats of Cyprus, overall and in relation with their lifestyle (indoor/outdoor) and the different districts [D1: Lemesos (n = 87), D2: Pafos (n = 64), D3: Lefkosia (n = 19), D4: Larnaka (n = 11), D5: Ammochostos (n = 4)]

Parasite	No of positive cats (% ± CI)	No of positive indoor/outdoor cats (%)	D1 positive (%)	D2 positive (%)	D3 positive (%)	D4 positive (%)	D5 positive (%)
Toxocara cati	22* (12 ± 4.6)	21/1 (11.3/0.5)	8 (9.2)	12 (18.7)	1 (5.3)	1 (9)	0 (0)
Cystoisospora rivolta	22* (12 ± 4.6)	20/2 (10.8/1)	11 (12.6)	8 (12.5)	1 (5.3)	1 (9)	1 (25)
Cestodes[a]	13* (7 ± 3.6)	12/1 (6.5/0.5)	6 (6.9)	5 (7.8)	0	1 (9)	1 (25)
Giardia spp.	12* (6.5 ± 3.5)	10/2 (5.4/1)	2 (2.3)	9 (14)	1 (5.3)	0 (0)	0 (0)
Troglostrongylus brevior	9* (5 ± 3.1)	9/0 (5/0)	7 (8)	0 (0)	1 (5.3)	1 (9)	0 (0)
Cystoisospora felis	5* (2.7 ± 2.3)	5/0 (2.7/0)	1 (1.1)	2 (3.1)	0 (0)	2 (18)	0 (0)
Aelurostrongylus abstrusus	4 (2 ± 2)	4/0 (2/0)	0 (0)	4 (6.2)	0 (0)	0 (0)	0 (0)
Dipylidium caninum	1 (0.5 ± 1.4)	1/0 (0.5/0)	0 (0)	1 (1.5)	0 (0)	0 (0)	0 (0)
Taenia spp.	1* (0.5 ± 1.4)	1/0 (0.5/0)	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)
Total	66 (35.7 ± 6.9)	60/6 (32.4/3.2)	25* (28.7)	32* (50)	3* (15.8)	5* (45.6)	1* (25)

[a] *Joyeuxiella*/*Diplopylidium* spp.

*Includes co-infections: parasites (no. of animals): *T. cati* + *C. rivolta* (3), *C. rivolta* + cestodes (3), *T. cati* + *T. brevior* (3), *T. cati* + *Giardia* spp. (2), *T. cati* + cestodes (1), *T. cati* + *C. felis* (1), *C. rivolta* + *T. brevior* (1), *C. felis* + *T. brevior* (1), *T. cati* + *C. felis* + *C. rivolta* (1), *T. cati* + *C. rivolta* + cestodes (1), *T. brevior* + *Taenia* spp. + *T. cati* + *C. rivolta* + cestodes (1)

Discussion

The results of the present study constitute the first record of feline pulmonary and intestinal parasites in Cyprus. At least one parasite was detected in 66 out of the 185 cats examined. This prevalence of parasitism is very close to that shown in two recent multicentre European surveys (Beugnet et al. 2014; Giannelli et al. 2017), where the percentage of infected cats varied between 30.8 and 35.1%. The statistically significant ($p < 0.05$) higher prevalence of infection in the cats of Pafos could be attributed to the fact that a large proportion of the cats examined from that district were living in shelters, receiving deworming treatment only sporadically.

Among the parasites found here, three, i.e. *T. cati*, *D. caninum* and maybe also *Giardia* spp., depending on the assemblages of the parasites, are of zoonotic interest, as they may infect and cause clinical conditions in humans (Chappell et al. 1990; Baneth et al. 2016).

Toxocara cati, one of the most common feline endoparasites, as confirmed in a recent survey in Europe where it was found in 19.7% of the cats (Beugnet et al. 2014), was also the most prevalent helminth here detected (12%). The high level of infection by *T. cati* in cats globally is usually due to its direct life cycle, lactogenic transmission, the role of paratenic hosts that may be preyed by outdoor cats and the high environmental resistance of eggs (Traversa 2012). It is worth mentioning that although this parasite is more prevalent in kittens and young cats (Beugnet et al. 2014) age was not associated with infection by *T. cati* in the present survey. Although common, the presence of *T. cati* is of great importance, as it may cause clinical disease in infected animals (Overgaauw and Nederland 1997) and, importantly, may cause larva migrans syndromes in humans (Despommier 2003; Holland and Smith 2006; Lee et al. 2010). Human toxocarosis is among the most common parasitic zoonoses worldwide (Rubinsky-Elefant et al. 2010), and it is estimated that the prevalence of infection in humans may be up to 5 and 42% in urban and rural areas, respectively, even in developed countries (Moreira et al. 2014).

Coccidial infection (*Cystoisospora* spp.) was the second most prevalent (overall 14.5%) parasitosis in cats of Cyprus, as also previously described (Beugnet et al. 2014; Diakou et al. 2017; Szwabe and Blaszkowska 2017). *Cystoisospora* spp. are protozoan parasites with direct life cycle, easily transmitted via contaminated soil, occasionally causing diarrhoea, anorexia, weight loss, vomiting and even death, especially in kittens (Greene 2013).

Infection by cestodes (overall 8%) reveals the potential of the cats to prey on, or incidentally ingest, intermediate (arthropods) and paratenic hosts (reptiles or rodents) in the case of *Joyeuxiella* spp., *Diplopylidium* spp. and *Taenia* spp., or fleas, in the case of *D. caninum*. Cestode infections are often underestimated when investigated by faecal examinations, due to the intermittent occurrence of proglottids and eggs (Dantas-Torres and Otranto 2014). Also, these infections are usually subclinical, though in some cases they induce non-specific digestive clinical signs, such as constipation, diarrhoea or even intestinal obstruction and intestinal pleating (Papazoglou et al. 2006; Bowman et al. 2002). Human dipylidiosis, described mostly in young individuals (rev. by García-Agudo et al. 2014), occurs by unintended ingesting of infected fleas or parts of fleas. Similarly to animals, the infection often remains subclinical or it is occasionally accompanied by diarrhoea or/and constipation, abdominal colic, rectal itching (caused by the emerging proglottids), eosinophilia and allergic manifestations (rev. by García-Agudo et al. 2014).

The flagellate protozoan parasite *Giardia* spp., found in 6.5% of the cats in Cyprus, is rather common in animals, and the prevalence of infection in cats is estimated to be around 12% (Bouzid et al. 2015). Giardiosis can be often subclinical, but sometimes diarrhoea, steatorrhea, vomiting, anorexia and weight loss are observed (Ballweber et al. 2010). According to genotyping investigations, *Giardia* isolates are classified into different assemblages (Ballweber et al. 2010). Although assemblages A and B have been isolated both from animals (dogs and cats) and humans, and thus considered zoonotic (Thompson et al. 2008), it is still unclear to what extent, if at all possible, a zoonotic transmission of *Giardia* spp. occurs. For example, most of the household-based molecular epidemiological surveys that have been published so far show limited or no indications of transmission from pets to humans or vice versa of *Giardia* spp. (reviewed by de Lucio et al. 2017). Moreover, further validation of observations regarding common assemblages in animals and humans are required by multiple locus sequence analysis and by additional longitudinal studies from animals and humans in the same area and time period, in order to unequivocally conclude that these assemblages represent zoonotic agents (Ballweber et al. 2010; de Lucio et al. 2017).

Two lungworms, i.e. *A. abstrusus* and *T. brevior*, were found in the cats examined in Cyprus at a prevalence of 2 and 5%, respectively. *Aelurostrongylus abstrusus* is renowned as the "cat lungworm", for its worldwide distribution (Traversa and Di Cesare 2016). It is noteworthy that in the present study, all infected by *A. abstrusus* animals had an outdoor lifestyle, thus confirming the importance of free-roaming animals in maintaining the infection, as these cats are more at risk of ingesting infective third-stage larvae (L3) harboured by gastropod intermediate hosts or by small animals (rodents, reptiles and amphibians), that serve as paratenic hosts (Gerichter 1949; Di Cesare et al. 2013).

The finding of *T. brevior* in the present study represents an extension of the known distribution of this parasite, currently reaching its easternmost border of occurrence in domestic cats in Cyprus. Interestingly, *T. brevior* has been found before on six different Mediterranean islands, i.e. Ibiza, Sardinia, Sicily, Crete, Mykonos and Skopelos (Jefferies et al. 2010; Brianti et al. 2013; Diakou et al. 2014; Tamponi et al. 2014; Diakou et al. 2015). It has also been reported mainly in Apennine areas of Italy (Di Cesare et al. 2015a), while the report from continental Greece was the first recording of the parasite in the Balkan Peninsula (Diakou et al. 2015). More recently, *T. brevior* has also been found in more areas of Greece (unpublished data, Fig. 1), in Bulgaria and continental Spain, but it has not been found in Central and Northern Europe (Giannelli et al. 2017).

The notion that *T. brevior* has a higher prevalence and wider distribution among domestic cats than previously estimated (Brianti et al. 2012) has been confirmed with a series of recent findings (Tamponi et al. 2014; Di Cesare et al. 2015a, d; Diakou et al. 2015; Giannelli et al. 2017). However, the enigma of *T. brevior* as a parasite of domestic cats, after its first unequivocal description in this animal species in Ibiza (Jefferies et al. 2010) remains unsolved, due to a lack of ultimately convincing scenarios for its interpretation. For example, the hypothesis of a frequent misdiagnosis (erroneous diagnosis of aelurostrongylosis instead of troglostrongylosis) is rational, but several data make it unlikely (Traversa and Di Cesare 2013). It is true that *A. abstrusus* and *T. brevior* share the same biological features and ecological niches (molluscs and small vertebrates as intermediate and paratenic hosts, respectively) (Gerichter 1949; Di Cesare et al. 2015e), that their L1 (diagnostic stage) are morphologically similar (Brianti et al. 2014), and that they cause overlapping clinical signs (Traversa and Di Cesare 2013). However, these parasites are also distinct in several ways. *Aelurostrongylus abstrusus* is located in the terminal bronchioles, alveolar ducts and alveoli and *T. brevior* in the large bronchi and trachea (Brianti et al. 2012). Moreover, *T. brevior* alone is to be found in kittens just a few weeks old (possible vertical transmission) (Brianti et al. 2012; Diakou et al. 2014) and the clinical infection in young animals is most often more severe, even life-threatening, when caused by *T. brevior* (Traversa and Di Cesare 2013). Finally, there are distinct gross lesions described for each of these parasitoses (Brianti et al. 2012).

A different approach to understanding the occurrence of *T. brevior* in domestic cats is the hypothesis that a spill-over occurs under specific environmental conditions from the natural host, the European wildcat (*Felis silvestris silvestris*), to domestic cats (Traversa and Di Cesare 2013, 2014; Traversa 2014). This scenario is supported in many cases, where the occurrence of the parasite follows the distribution of wildcat populations (Diakou et al. 2014; Di Cesare et al. 2015d,e). Nevertheless, there have been reports of the parasite in areas (Ibiza, Mykonos, Skopelos and Athens), where wildcats are not present (Jefferies et al. 2010; Diakou et al. 2015). Similarly, wildcats are non-existent in Cyprus, and moreover, according to paleontological surveys, there is no evidence of any native felid species in this country. On the contrary, there is evidence of tamed cats on the island starting from around 9500 years ago (Vigne et al. 2004). Thus, the most realistic scenario seems to suggest that *T. brevior* was introduced to the island along with the domestic cat sometime in the past and later has become enzootic, as some of its intermediate hosts, i.e. molluscs of the genera *Helix*, *Limax*, *Theiba* (Gerichter 1949; Giannelli et al. 2014), are present on the island (Cowie 1984; Lazaridou-Dimitriadou et al. 1994; Vardinoyannis et al. 2012). Accordingly, the genetic haplotype (haplotype II) found here is one of the most commonly

Fig. 1 Map of documented distribution of *Troglostrongylus brevior* in domestic cats, including previous reports (stars), unpublished data (triangles) and the findings in Cyprus (dot)

found haplotypes in various locations, in both domestic and wildcats, including Mykonos in Greece (Traversa et al., 2017).

Although climate changes have been suggested to concur to geographic expansion of mollusc-transmitted parasitoses (Traversa et al. 2010), it is unlikely that the increasing number of reports of troglostrongylosis is due only to changes in climate and mollusc phenology. In fact, *T. brevior* L1 are able to reach the infective stage after 40 days at 4–8 °C, whilst *A. abstrusus* L1 remain totally arrested, even after 7 months in these conditions (Gerichter 1949). Knowledge on the geographic distribution of troglostrongylosis is still incomplete, and according to the biological feature mentioned above, *T. brevior* should have a wider distribution than *A. abstrusus* towards the north of Europe. Nonetheless, the opposite situation has been observed so far: although the northernmost report of *A. abstrusus* to date is Sweden (Grandi et al. 2017), *T. brevior* was only found to date as far north as Northern Italy (Di Cesare et al. 2015e).

Alongside the abovementioned debated reasons for the increasing occurrence of *T. brevior*, greater awareness in the scientific community that has increased accurate diagnosis as well as the development of highly evolved diagnostic tools, such as PCR, that allow accurate and refined identification of the parasites should also be taken into consideration.

It is noteworthy that *T. brevior* was found here mostly in < 1-year-old cats, with a statistically significant association ($p = 0.04$) that could either be attributed to a possible vertical transmission (Brianti et al. 2013; Diakou et al. 2014) and/or an easier establishment in younger cats (Traversa and Di Cesare 2013). Interestingly, in the present survey, three 3-month-old stray kittens of the same litter were shedding *T. brevior* L1. This finding supports the hypothesis that entire litters may get the infection from the queen, although it is also possible that these kittens were living in an infected environment and another route of infection occurred. At present, occurrence and mechanisms of potential vertical transmission for *T. brevior* require further confirmation and further studies and reports are thus advocated.

In conclusion, these results reveal for the first time in the cats of Cyprus a variety and a noteworthy prevalence of endoparasites, which was over tenfold higher in the cats with an outdoor lifestyle. Routine parasitological examinations and antiparasitic treatment are imperative measures to ensure the good health of cats and in favour of public health protection from zoonotic parasites. This was clearly emphasised by the statistical analysis of the results that showed a significantly lower prevalence of infection in animals with a history of recent deworming treatment. Moreover, insisting on the continuation and extension of epizootiological surveys in pursuit of newly recognised feline parasites like *T. brevior* may offer useful information regarding their occurrence, prevalence, distribution range and life cycle. Finally, veterinarians should be vigilant against the presence of respiratory signs in cats and larvae in their faeces and be aware of parasiticide molecules efficacious for lungworms (especially *T. brevior*), e.g. emodepside, eprinomectin and moxidectin (Crisi et al. 2015; Di Cesare et al. 2015b; Giannelli et al. 2015).

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