



Epidemiological survey on intestinal helminths of stray dogs in Guimarães, Portugal

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Abstract The new legislative framework on Animal Welfare brought increased responsibilities to municipal shelters, in particular in the collection of stray dogs, their sterilization and future adoption. These centers quickly became overcrowded, leading to high parasitism environmental contamination, to the easy spread of parasitic infections and to increased risks to public health. The prevalence of intestinal parasites was evaluated by examination of dog faecal sample, in the municipal control animal centre of Guimarães (north Portugal), identifying risk factors and transmission to man. The overall prevalence of gastrointestinal helminths was 57.2% (95% confidence interval 41.3–71.9%) and observed helminths of the gastrointestinal tract were recorded: *Ancylostoma caninum* (33%), *Toxocara canis* (29%), *Dipylidium caninum* (6%), *Capillaria* spp. (3%), *Trichuris vulpis* (1.66%). It is important to point out that young dogs were significantly infected more frequently ($p \leq 0.1$) than non-sterilized females and the higher occurrence of nematode infection occurred at the new arrival of stray dogs, in the third collection. With impact on public health, the higher prevalence ($p \leq 0.1$) of *T. canis* in young dogs suggests the existence of real risk for human infection and demonstrate

the necessity for a parasite control programme reinforcement at the municipal dog shelter.

Keywords Animal shelters · Stray dogs · Nematodes · Risk factors · Public health

Introduction

Parasitic diseases are often underestimated in the eyes of civil, medical and scientific society, but they are of utmost importance, and comparable to infectious diseases. In Portugal exists an increasing number of stray animals, both in urban and rural areas, where, during 2018, more than 40 thousand stray animals were collected from the streets and, in 2019, in an estimated population of 891.788 dogs, only 539.025 were identified and registered (DGAV 2019; FECAVA 2019).

This represent a substantial Public Health risk factor, from an epidemiological point of view, due to the strong environmental contamination of stray dog's feces, without any deworming program and with a high probability of carrying pathogens and parasites, easily transmitted to man. As a potential reservoir of endoparasites, the contamination of public places increases the susceptibility of others animals and humans, in a distressing zoonotic chain (Dado et al. 2012).

“One Health” is a worldwide strategy whose purpose is the expansion of interdisciplinary communications, which link human, animal and environmental health. Not all the mentioned categories are independent and, in fact, parasitism in pets, directly affects the community health, either through direct or environmental contact. It is critical that the control, both of internal and external parasites, occurs

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periodically, avoiding the parasitic zoonosis transmission (Neto and Coelho 2016).

As an animal shelter, the Control Animal Centre (CAC) has an important role in Public Health in the collection of stray animals, in their sterilization, in the implementation of medical and prophylactic control measures, as deworming, and in the promotion of adoption policies. In 2018, the law that forbids the euthanizing of pets due to economic incapacity or overcrowding was enacted (DRE 2019). However, the law brought many inconveniences to the municipalities and the number of stray dogs has been progressively rising, leading to the overpopulation and the reduction of housing conditions. There are, in most cases, several animals living in tiny cages, inducing higher parasitism prevalence (Bresciani et al. 2013), especially when new animals arrive.

In view of the high number of parasites with zoonotic potential, the awareness of the society is extremely important, as well as the correct provision of information to future guardians seeking to adopt animals at the CAC. This lack of information generates great concern, since naivety in relation to the topic promotes an inappropriate use of deworming which, consequently, induces the parasitism resistance and inefficiency in parasite load elimination (Pereira et al. 2016).

The most commonly detected dog parasites of the gastrointestinal tract are *Dipylidium caninum*, *Toxocara canis*, *Ancylostoma* spp., *Trichuris vulpis*, *Taenia* spp. and *Cystoisospora* spp. (Mateus et al. 2014). Although less frequent, *Hammondia heydorni* and *Cryptosporidium* spp. are also observed as well as *Neospora caninum*, a mandatory intracellular protozoan, is frequently found in the blood system (Funada et al. 2007; Taylor et al. 2016).

There are no specific studies about parasitism in stray dogs gathered in official shelters, although a higher prevalence of *A. caninum*, *T. canis*, *T. vulpis*, *Cryptosporidium* spp. and *Strongylidium* spp. in Portugal was reported (Silva 2010; Melo and Lebre 2011; Otero et al. 2014). The main objective of this study is to determine the prevalence of gastrointestinal helminthes in stray dogs in Guimarães, with especial attention to potential zoonotic diseases.

Methodology

Sampling

This epidemiological study was carried out based on the collection of dog's faecal samples from the Guimarães CAC, a municipal shelter. The CAC has a maximum accommodation capacity of 100 animals and perform the sterilization and the adoption of stray animals. An external

and internal deworming plan is established, consisting in the administration of Milbemycin oxime and Praziquantel, in an age frequency dependence.

The study, outlined for 1 year but interrupted by the COVID-19 pandemic, occurred for a period of 3 months, from November 2019 to January, 2020 when the CAC average population was 78 animals. A sample of 25% of the animals was considered and divided into the three CAC groups: young dogs (under the age of 1 year); sterilized males and females (over the age of 1 year); and non-sterilized females (over the age of 1 year). Feces, 7 samples from each group, were collected every 3 weeks, stored at 4 °C and processed within 24 h in the Department Laboratory.

Parasitological procedures

Fecal samples were first examined for macroscopic observation and, following, faeces were processed using the techniques of concentration by sedimentation (Ritchie 1948) and flotation by centrifugation-flotation (MAFF 1986; Bowman et al. 2004) and counted in a McMaster chamber (Vasconcelos-Nóbrega et al. 2017). Identification of eggs and larvae was based according to the literature keys and guidelines (Yamaguti 1961; Menezes et al. 2013).

Statistical methodology

Analyses were performed with SPSS®22 software for Windows. Pairwise comparisons between categories of the same independent variable incorporated Bonferroni's correction. A p value ≤ 0.1 was considered as statistically significant.

Results

Macroscopic analysis results are presented in Table 1. Only 15 of the 63 total samples presented a non-normal consistency (6 liquids and 9 pasties), and relatively to color, 15 samples had a light brown color, including 6 samples with undigested food particles. No parasites adult forms were observed.

Feces with a pastier consistency were identified (Table 1), more evidently in the third collection and, in particular, in younger animals. In this specific group, was registered color change (light brown) and the presence of poorly digested foods.

Concerning the microscopic analysis (Table 2), several eggs parasitic forms were identified. The overall prevalence of parasitic infection with parasites was 57.2% [(95% confidence interval (CI) 41.3–71.9%)] and the most frequently observed parasite was *Ancylostoma caninum*

Table 1 Macroscopic analysis—collections qualitative results

| Collection 1 | Young dogs | | | | | | | | | | | | | | | | | | | | | |
|--------------|------------|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|----|
| | SMF | | | | | | | NSF | | | | | | | | | | | | | | |
| | YD1 | YD2 | YD3 | YD4 | YD5 | YD6 | YD7 | SMF1 | SMF2 | SMF3 | SMF4 | SMF5 | SMF6 | SMF7 | NSF1 | NSF2 | NSF3 | NSF4 | NSF5 | NSF6 | NSF7 | |
| Consistency | N | N | N | N | N | N | N | L | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Colour | B | B | B | B | B | B | B | LB | B | B | B | B | B | B | B | B | B | B | B | B | B | B |
| Composition | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Collection 2 | YD1 | YD2 | YD3 | YD4 | YD5 | YD6 | YD7 | SMF1 | SMF2 | SMF3 | SMF4 | SMF5 | SMF6 | SMF7 | NSF1 | NSF2 | NSF3 | NSF4 | NSF5 | NSF6 | NSF7 | |
| Consistency | N | N | N | N | N | N | N | L | L | L | N | N | N | N | N | N | N | N | N | N | N | N |
| Colour | B | B | B | B | B | B | B | LB | LB | LB | B | B | B | B | B | B | B | B | B | B | B | B |
| Composition | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |
| Collection 3 | YD1 | YD2 | YD3 | YD4 | YD5 | YD6 | YD7 | SMF1 | SMF2 | SMF3 | SMF4 | SMF5 | SMF6 | SMF7 | NSF1 | NSF2 | NSF3 | NSF4 | NSF5 | NSF6 | NSF7 | |
| Consistency | P | P | P | P | P | P | P | N | L | L | N | N | P | P | N | N | N | N | N | N | N | P |
| Colour | LB | LB | LB | B | LB | LB | LB | B | LB | LB | B | B | LB | LB | B | B | B | B | B | B | B | LB |
| Composition | UF | UF | UF | N | UF | UF | UF | N | N | N | N | N | N | N | N | N | N | N | N | N | N | N |

YD young dogs, SMF sterilized males and females, NSF non sterilized females, N normal, L liquid, B brown, LB Liquid brown, P pasty, UF undigested food

Table 2 Microscopic analysis—quantitative results (faecal eggs count, FEC)

| Group | n | n/ prevalence | <i>Toxocara canis</i> | | <i>Toxocara canis</i> (L2) | | <i>Ancylostoma caninum</i> | | <i>Capillaria</i> spp. | | <i>Trichuris vulpis</i> | | <i>Dipylidium caninum</i> | |
|-------|---|------------------|-----------------------|-------------------------------------|-------------------------------|-------------------------------------|----------------------------|-------------------------------------|------------------------|-------------------------------------|-------------------------|-------------------------------------|---------------------------|-------------------------------------|
| | | | Eggs (FEC) | n infected animal/ prevalence | L2 | n infected animal/ prevalence | Eggs (FEC) | n infected animal/ prevalence | Eggs (FEC) | n infected animal/ prevalence | Eggs (FEC) | n infected animal/ prevalence | Eggs (FEC) | n infected animal/ prevalence |
| YD1 | 7 | 4 57.10 | – | – | – | – | 4 | 4 | – | – | – | – | – | – |
| YD2 | 7 | 7 100 | 205 | 7 100 | – | – | 1 | 1 | – | – | – | – | – | – |
| YD3 | 7 | 6 86 | 3 18 | – 43.00 | – | – | 1146 | 6 86 | 1 | 1 14.30 | 1 | 1 14.30 | 95 | 1 14.30 |
| SMF1 | 7 | 2 28.60 | 1 | – 14.30 | – | – | 4 | 2 28.60 | – | – | – | – | – | – |
| SMF2 | 7 | 4 57.10 | 2 19 | – 28.60 | – | – | 3 | 2 28.60 | – | – | – | – | – | – |
| SMF3 | 7 | 5 71.40 | 1 4 | – 14.28 | – | – | 100 | 3 42.84 | – | – | – | – | 223 | 2 28.60 |
| NSF1 | 7 | 2 28.60 | 1 33 | – 14.30 | 2 | 1 14.30 | 16 | 1 14.30 | – | – | – | – | – | – |
| NSF2 | 7 | 4 57.10 | 2 8 | – 28.60 | – | – | 1 | 1 14.30 | 1 | 1 14.30 | – | – | – | – |
| NSF3 | 7 | 2 28.60 | 1 6 | – 14.30 | – | – | 21 | 1 14.30 | – | – | – | – | 11 | 1 14.30 |

YD young dogs, SMF sterilized males and females, NSF non sterilized females

(33%), followed by *Toxocara canis* (29%) and *Dipylidium caninum* (6%).

In line with the macroscopic analysis results, an important increase in parasitism in the third collection was verified, particularly in younger animals (86% prevalence), but also in sterilized ones (71.4% prevalence). At this third sampling, *A. caninum* (present in all samples) and *T. canis* revealed a higher prevalence (86% and 43%, respectively), in terms of group and/or reproductive status.

In addition to the high parasite load verified (particularly, *A. caninum* 1146 FEC) in the third collection, the presence of eggs of *Trichuris vulpi* (1FEC), *Capillaria* spp. (1FEC) and *D. caninum* were identified also mainly in young dogs. The presence of *D. caninum* (329 FEC) was observed, only in this collection, in all groups analyzed, but particularly in sterilized animals (29% prevalence). Regarding the first collection, a second-form L2 larvae of *T. canis* was identified in the group of non-sterilized females. Also in this group, but in the second collection, eggs of *Capillaria* spp. were observed in a small infestation (1FEC).

The mean and standard deviation parasite load values (FEC) obtained by group and collection are observed in Fig. 1, stressing the high values verified in the youngest animals and third collection, comparing to the lowest levels

of adult animals' infestation (sterilized or not), with the exception of the third SMF sample.

Significant differences ($p \leq 0.1$) were found among collections (Table 3), with higher values between the third (73.190 FEC), the first (2.143 FEC) and the second (10.762 FEC) collection, as well as groups with higher ($p \leq 0.1$) parasite load value in the youngest (66.81 FEC) comparing to the NSF (3.38 FEC).

The average values (FEC) per group, were estimated and higher parasite load of *T. canis* (10.62 ± 15.66 FEC) and *D. caninum* (55 ± 156.57 FEC) was found in younger animals while *A. caninum* (4.52 ± 20.271 FEC) showed higher levels in sterilized animals. *T. canis* also presented significant differences between groups ($p \leq 0.05$), with a superior infestation (10×) in the youngest animals (10.62 FEC) comparing to the sterilized (1.14 FEC) and non-sterile (1.71) groups.

Discussion

Natural transmission of parasitic infections from dogs to man may occur, directly or indirectly, via environmental factors and represent a potential public health risk, particularly to individuals with close contact with those animals

Fig. 1 Mean parasite load (FEC), considering group and collection



Table 3 Statistic mean differences ($p \leq 0.1$) between collections and groups

| (i) Collection/ mean (FEC) | Difference of means (I–J) | Sig. | Confidence interval 90% | | (ii) Group/mean/ FEC) | Difference of means (I–J) | Sig. | Confidence interval 90% | |
|-------------------------------|------------------------------|-------|----------------------------|----------------|--------------------------|------------------------------|------|----------------------------|----------------|
| | | | Lower limit | Upper limit | | | | Lower limit | Upper limit |
| Dec 10.76 | Jan – 62.43* | 0.059 | – 126.69 | 1.84 | NSF 3.38 | SMF – 12.52 | 0.89 | – 68.44 | 43.39 |
| | Nov 8.62 | 0.944 | – 55.65 | 72.89 | | YD – 63.43* | 0.05 | – 119.34 | – 7.51 |
| Jan 73.19 | Dec 62.43* | 0.059 | 1.84 | 126.7 | SMF 15.91 | NSF 12.52 | 0.89 | – 43.39 | 68.44 |
| | Nov 71.05* | 0.027 | 6.78 | 135.31 | | YD 50.9 | 0.15 | – 106.82 | 5.01 |
| Nov 2.14 | Dec – 8.62 | 0.944 | – 72.89 | 55.65 | YD 66.81 | NSF 63.43* | 0.05 | 7.51 | 119.34 |
| | Jan – 71.05* | 0.027 | – 135.31 | – 19.3 | | SMF 50.9 | 0.15 | – 5.01 | 106.82 |

*Difference of means significant on level 0.10

(Martínez-Moreno et al. 2007). CAC are an important indicator for the evaluation of regional parasite prevalence and, consequently, develop a relevant role in animal and public health.

The overall prevalence of intestinal parasitosis found in this study is 57.2%, revealing a considerable presence of gastrointestinal parasites in stray and shelters dogs, compared to the lower prevalence values observed in other Europeans countries (Pullola et al. 2006; Becker et al. 2012; Dado et al. 2012; Zanzani et al. 2014). Similar values (54.3%) were found by Katagiri and Oliveira-Sequeira (2008) in stray and domiciled dogs in Brasil, also in stray dogs in Nigeria (52.6%, Okoye et al. 2011), Malaysia (48%, Mahdy et al. 2012) and Canada (21%, Joffe et al. 2011). Higher prevalence was reported, in stray dogs, in Iran (86%, Emamapour et al. 2015), México (85%, Eguía-Aguilar et al. 2005), South Africa (76%, Minner et al. 2002), Spain (71%, Martínez-Carrasco et al. 2007) and Poland (68%, Bajera et al. 2011).

Our results indicate the requirement, in the official dog shelters, of an effective anti-parasite control programme, due to the continuous collection of stray dogs, with no

health control measures and, because of their habits, exposed to natural infections. Shelter dogs are exposed to a greater parasite load, to less effective anti-parasite treatments, and have a poorer nutritional status. This implies a change in shelter’s routine, such as the entry of new animals, particularly pups, which has a direct influence on the increase in the parasite load and requires more frequent hygiene and prophylaxis measures, as quarantine or deworming.

Pups are infected by vertical transmission, transplacental and/or trans-mammary as well as horizontal transmission through the ingestion of embryonated eggs from the environment or ingestion of larvae via vertebrate and/or invertebrate paratenic hosts (Overgaauw and van Knappen 2013). The *T. canis* trans-placental infection route results in egg excretion 16 days after parturition and lactogenic transmission, more limited, continues to occur for 5 weeks, shedding, after infection, millions of eggs per day into the environment (Lloyd and Morgan 2011).

In fact, parasitic infections were observed specially in the third collection, in young animals that had recently arrived and were dewormed, revealing a severe

environmental contamination with a higher risk of zoonotic transmission from dogs. Given this prevalence in pups, specific interventions with a focus in animal birth control, parasites control programs and public education to take wise action relating to the parasites and pets, need to be reinforced. Moreover, animal shelters facilitate spread of gastrointestinal parasites to incoming animals and shelter staff if there is overcrowding and frequent exposure to a contaminated environment (Raza et al. 2018).

According to similar results in different studies, the most prevalent species observed were *A. caninum*, and *T. canis*, widely known as potential zoonotic agents (Emamapour et al. 2015; Cociancic et al. 2018; Suganya et al. 2019). *A. caninum* is the primary hookworms' specie infecting dogs worldwide (Traversa et al. 2014). Hookworms may occur in dogs of all ages, independently of sex or season (Coggins 1998), even when under regular control programs (Sager et al. 2006), as verified in our study, where no significant difference were obtained between groups.

The zoonotic importance of *A. caninum*, a historical association of humans, dogs and hookworms, resides in that its larvae survive in the environment for several months (Shepherd et al. 2018). Adult dogs can become infected with environmental larvae or when hypobiotic stages are re-activated by drivers of stress (Bowman et al. 2004). Hypobiotic larvae may survive for years in the tissues of adult dogs and when reactivated during oestrus and in the last 2–3 weeks of pregnancy, transmitted via milk to the litter (Little et al. 2009; Traversa et al. 2014).

Although has been decreased significantly over time, presumably due to routine use of broad-spectrum anthelmintics, *T. canis* is the primary roundworm specie infecting dogs worldwide (Robertson and Thompson 2002). Eggs of *T. canis* are very resistant and can withstand harsh environmental conditions and is estimated that *Toxocara* eggs contamination soil may be more than the 90% of the investigated areas worldwide (Kirchheimer and Jacobs 2008).

T. canis is more prevalent in puppies and can be fatal, especially when there is heavy prenatal infection (Overgaauw 1997). Pups are infected in utero by reactivated somatic larvae of *T. canis* from the mother from day 42 of gestation resulting in egg excretion ~ 16 days after parturition (Traversa et al. 2014). Puppies can also be infected through the transmammary route (Robertson and Thompson 2002). Once infected, pups shed millions of eggs per day into the environment, depending on the intensity of *T. canis* infection and host immune status (Glickman and Schantz 1981).

In our study, significant differences were observed between young and adult dogs infected with *T. canis*. These results are consistent with other findings revealing

that adult worm infections are generally less common in dogs with less than 6 months of age, and that faecal egg counts are much lower than in pups (Papazahariadou et al. 2007; Xhaxhiu et al. 2011).

D. caninum is the most common cestode in the world, especially in dogs, frequently infested by fleas and biting lice and with a higher prevalence in adult animals (Raza et al. 2018). Humans can become infected and very young children are the ones most often affected, associated with diarrhea and abdominal pain (Molina et al. 2003). The prevalence observed in this study is significantly lower than in others reported by different authors (Xhaxhiu et al. 2011; Emamapour et al. 2015) and was verified in all ages but only in the third collection, what can be attributed to an infestation by the eventual entry of new stray dogs.

Zoonotic parasitosis is a serious public health problem and parasitism in stray and shelter dogs is a relevant indicator of, both, the deficiencies in the sanitary and ecological conditions of their hosts, of the socio-environmental conditions and hygiene practices of the population (Cociancic et al. 2018). In order to reduce environmental contamination and parasitism in the shelter, the implementation of sanitation measures is mandatory, such as quarantine of new stray dogs collected or more regular faecal examination, and the appropriate use, particularly in terms of dose and frequency, of deworming agents, according to the animal's weight and the shelter parasitic load (Raza et al. 2018). Anthelmintic therapy should be accompanied by flea control programs to minimize transmission of *D. caninum* (Taylor et al. 2016).

The full development of our study was compromised by the COVID-19 pandemic, as it had the objective of lasting 1 year, evaluating diverse factors with influence on parasitism. The data about type and parasitic load and its influence in the community, are required to the implementation of public health policies, with the support of the veterinary health authorities.

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Author's contribution All authors contributed to the study conception and design. Collection samples and identification of the parasites were done by Vanessa Silva and Margarida Gonçalves, data analysis were performed by Joana Silva, Carlos Brandão. The first draft of the manuscript was written by Vanessa Silva. Carlos Brandão and Nuno Vieira e Brito reviewed the article. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Ethical approval Not applicable. Authors were not involved with animal handling and the fecal samples were obtained directly through the shelter staff, specifically from the veterinary shelter authority.

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