

Original Article

Prevalence, diversity and risk factors of gastrointestinal parasites in dogs housed at official shelters across Portugal

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ABSTRACT

Gastrointestinal parasites (GIP) are among the most common pathogens that affect dogs globally, with some species representing a zoonotic threat. Stray and shelter dogs are especially vulnerable to parasitism as their clinical history is often unknown, and copro-parasitological analyses are seldom performed upon animal arrival at shelters. In Portugal, official shelters play a pivotal role in sheltering and managing stray dogs; however, little is known about the GIP infection status of these animals. This study thus aimed to determine the occurrence of GIP in dogs housed at official shelters in Portugal and explore potential variables contributing to the GIP burden. Between November 2021 and October 2022, a total of 355 dog faecal samples were collected from shelters across the country. These samples were either obtained individually from dogs or pooled from groups of animals housed together, and were analysed using the Mini-FLOTAC technique with zinc sulphate flotation solution (specific gravity 1.35) to detect, identify, and quantify GIP shedding intensity. Over 16 % (57/355) of the samples were GIP-positive. The most frequently observed parasites were *Trichuris vulpis* (20/355), *Toxocara canis* (19/355), *Ancylostoma* spp. (13/355), and *Cystoisospora canis* (8/355). Co-infections with two parasitic agents were detected in three individual samples: two dogs harboured both *T. vulpis* and *C. canis*, while one dog was co-infected with *T. vulpis* and *T. canis*. Most positive samples (53/59) exhibited a moderate to high shedding intensity, with over 50 eggs or oocysts per gram of faeces - EPG or OPG, respectively. Only a few showed a low shedding intensity of less than 10 EPG or OPG. When examining individual faecal samples, the average prevalence rate of GIP in shelter dogs across sampled regions was 15.9 % (33/208). A single taeniid infection was detected and confirmed, via molecular assays, to be due to *Echinococcus granulosus*. The occurrence of *Ancylostoma* spp. and *T. vulpis* were significantly correlated with the number of dogs housed in shelters. The presence of *Ancylostoma* spp. eggs was significantly associated with season and region, while consistency of faeces was significantly associated with the presence of *T. canis* eggs and GIP co-infection status.

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This study represents the first nationwide screening for GIP in shelter dogs in Portugal. Our findings underscore the importance of regular screening in shelters, namely upon animals' arrival, and the need for active, effective management of infections to control GIP spread within the shelter and to prevent possible occupational zoonotic transmission.

1. Introduction

Gastrointestinal parasites (GIP) are commonly reported in dogs worldwide, with varying prevalences. Studies conducted on stray dog populations have reported prevalences ranging from 9.4 % in Germany (Becker et al., 2012) to 85.0 % in Mexico (Eguía-Aguilar et al., 2005). Numerous studies have investigated GIP in dogs across various regions of Portugal. Recent examples include surveys focusing on the northern (Silva et al., 2020), north-central (Rocha, 2022), and west-central (Ferreira et al., 2023) regions of the mainland territory. These regional studies highlight important findings but also underscore the need for a comprehensive, nationwide understanding of GIP prevalence in shelter and stray dog populations.

Routine screening for GIP in canine shelter populations serves multiple purposes: It generates essential epidemiological information regarding parasite distribution patterns, helps identify transmission risk factors within shelter environments, and enables the assessment of deworming protocol effectiveness. These screening programs allow early detection of potential anthelmintic (AH) resistance, which is important for safeguarding animal welfare and public health protection. Dogs harbouring resistant parasites who subsequently enter private homes through adoption potentially introduce these resistant organisms into the broader community (Raza et al., 2018).

Dogs in private households are generally well-cared for or tend to receive at least the fundamental care required, including routine prophylactic measures such as deworming treatments (Becker et al., 2012). Conversely, dogs housed in shelters may not receive previous care from committed and attentive caretakers (Becker et al., 2012). Before shelter admission, many of these animals may have experienced prolonged roaming periods, during which they might scavenge on carrion (Macpherson, 2005; Mateus et al., 2014) and discarded organic waste (Kardjadj and Ben-Mahdi, 2019); exhibit coprophagia (Nijse et al., 2014), a behaviour observed in both owned and stray dogs, though likely more prevalent in strays due to increased exposure to faecal material; and predate rodents, wild leporids and birds (Azevedo et al., 2023; Roldan and Otranto, 2023). These behaviours may expose stray dogs to zoonotic pathogens, in particular *Toxocara canis*, *Ancylostoma* spp. and *Echinococcus* spp., which are reported as the most prevalent and relevant to public health (Traversa et al., 2014; Casulli et al., 2022; Idrissi et al., 2022). The eggs of these parasites are then shed in the dogs' faeces and dispersed into the environment (Becker et al., 2012; Roldan and Otranto, 2023). Moreover, high-density populations, as may occur in multi-dog households and shelters amplify the risk of faecal contamination, posing a potential threat to both public and animal health unless effectively managed by rigorous sanitation measures (Liberato et al., 2018). Furthermore, shelter populations are especially challenging due to the unknown clinical history of incoming dogs, including records of vaccinations and antiparasitic treatments. Incoming dogs may be asymptomatic or already sick, malnourished, stressed, and carrying or shedding pathogens, including those with zoonotic potential (Traversa et al., 2017; Lopes et al., 2022; Afonso et al., 2024a). Consequently, if not appropriately managed, each new dog admitted to shelters may introduce pathogens into the housed populations and may expose shelter staff to zoonotic risk. Besides, incoming dogs may also acquire new infections inside these facilities. For this reason, GIP are a hazard to other animals and people, necessitating heightened surveillance and management within shelter environments (Neves et al., 2014; Traversa et al., 2017; Lopes et al., 2022). Furthermore, shelter dogs can serve as effective sentinels for zoonoses surveillance and are an essential

indicator of overall GIP prevalence (Traversa et al., 2017; Afonso et al., 2024b). Supplementary Table 1 presents a comprehensive overview of the reported prevalence of GIP found in dog faecal samples from other studies in Portugal and other countries. Diagnosing zoonoses in this specific population is, thus, crucial for planning control and preventive strategies at various administrative levels (i.e., local, regional, and country scales) (Traversa et al., 2017; Liberato et al., 2018; Afonso et al., 2023, 2024b).

The first national census of stray animals in Portugal estimated more than 100,000 stray dogs (Azevedo et al., 2023). Despite ongoing efforts to mitigate this issue, the population of abandoned animals demonstrates a persistent upward trajectory. This growing trend is underscored by data from the last five years, showing a 41.2 % increase in animal capture (DBEAC, 2024). Socioeconomic factors, such as lower family income values, have been associated with higher densities of stray animals in Portugal (Azevedo et al., 2023). In the country, municipal councils are the governing bodies responsible for collecting, capturing, and housing stray animals found in public domains. These dogs are housed in official shelters. However, municipal authorities often face challenges arising from shelter overpopulation and constrained resources (Afonso et al., 2023, 2024b). Given this overall context, copro-parasitological surveys of stray and shelter dogs are key to managing these particularly vulnerable populations and controlling transmission of endemic, emergent, or re-emergent GIP. With this in mind, this work aimed to conduct a nationwide screening of GIP in dogs at official shelters and identify potential variables related to the occurrence of detected GIP, with an emphasis on zoonotic parasites.

2. Methods

2.1. Sampling and processing of dog faecal samples

From November 2021 to October 2022, a total of 355 faecal samples from dogs were collected, comprising 208 individual samples and 147 pooled group samples from 19 official shelters serving 60 out of the 308 Portuguese municipalities. The shelters were geographically distributed across the following level II (i.e., main regions) of the Nomenclature of Territorial Units for Statistics (NUTS II) developed by Eurostat, the statistical office of the European Union: five in the North, five in the Centre, one in the Lisbon Metropolitan Area, five in the Alentejo, one in the Algarve, and two in the island of S. Miguel, Autonomous Region of Azores.

Before participating in the study, local authorities, including municipal veterinarians and councillors responsible for the official shelters, were provided with a detailed explanation of the study's objectives, along with the guarantee of anonymity and confidentiality. Accompanying these explanations were the principal researcher's professional identification and contact details. Faecal sample collection was initiated only after obtaining informed consent from these local authorities, ensuring compliance with regulatory requirements and ethical principles, and reflecting a commitment to transparency and ethical conduct. Veterinarians responsible for the official shelters randomly collected fresh faecal samples from the shelter grounds, ensuring only portions not in direct contact with the soil were taken to avoid contamination. In enclosures housing multiple dogs, composite samples were obtained by pooling small portions of faeces from different spots within the enclosure to ensure animal representation and minimize the risk of repeatedly sampling the same individual. Given the study's non-invasive nature, faecal samples were opportunistically collected from

the ground without any interaction or handling of animals that could cause stress or harm. This ensured no disturbance to the animals, aligning with ethical considerations for animal welfare. Additional welfare or ethical issues were not raised.

A form was utilised to gather metadata characterising the shelter, including the number of animals housed, recent variations in intake rate, pest occurrence, and deworming practices. Additionally, the form solicited sample-specific information, including the sampling date, number of dogs per enclosure (defined as housing units that can accommodate either individual animals or small groups), source of the animals, sex, estimated age range, and the last anthelmintic (AH) treatment date. The samples were kept on site at 4 °C for a maximum of 24 h until shipment to the Centre of Ecology, Evolution, and Environmental Changes at the Faculty of Sciences, University of Lisbon, Portugal. The samples were transported following triple packaging guidelines, which included the use of plastic sealed containers (primary packaging), zip-lock bags with absorbent material (secondary packaging), and a tertiary container with cooling elements to ensure that samples remained at 4 °C during transport. Upon arrival at the laboratory, the faecal samples were stored at 4 °C and processed within 24 h using copro-parasitological methods.

2.2. Official shelters' characterization

A total of 19 shelters, comprising both municipal and inter-municipal shelters (i.e., serving multiple municipalities), participated in the study, with two inter-municipal shelters representing eleven and thirteen municipalities, respectively. The survey encompassed a geographic area spanning 60 of the 308 municipalities in Portugal (INE, 2023). The number of dogs housed at surveyed shelters varied, ranging from 20 to 183, with a mean of 56 dogs per shelter. However, one shelter located in central Portugal housed over 600 dogs; this outlier was excluded from the mean calculation. Approximately 47.4 % of the shelters included in this study housed between 20 and 40 dogs at the time of sampling. Eleven shelters offered an average of 6 m² of space per animal. The remaining eight shelters had variable space allocation based on available enclosures for new entries. On average, two dogs were housed per enclosure, with a maximum of nine dogs in an 8 m² enclosure.

Most shelters (63.2 %) currently rescue many dogs from the streets. This dog rescue rate was assessed without specific comparative reference to previous years, serving to gauge the current volume of abandoned dogs entering the shelters. Three shelters that did not report high intake rates for incoming dogs mentioned reaching their maximum capacity for new arrivals as the reason for their inability to accommodate more dogs. The remaining respondents did not disclose the reasons for not having high incoming dog rates.

Only 26.3 % (five out of 19) of the shelters reported pest issues, with one reporting flea infestation, another reporting cockroach presence, and three reporting rodent infestations.

All shelters had implemented deworming protocols, with all newly arrived dogs receiving deworming treatment. The deworming medication generally contained cestocidal substances, except for one shelter that employed ivermectin (macrocyclic lactone) for deworming upon entry. Specifically, except for one shelter that employed only ivermectin at entry, all others reported the use of broad-spectrum products containing praziquantel (prazinoisoquinolone), with some (36.8 %) employing a combination of praziquantel with pyrantel (tetrahydropyrimidine) and a third molecule (febantel, a pro-benzimidazole, or fenbendazole, a benzimidazole). Five veterinarians indicated that they alternate between different drug combinations, always including praziquantel in their protocol. Among this subset of veterinarians, three rotated between broad-spectrum drugs that invariably included a benzimidazole as one of the active ingredients. One veterinarian limited the rotation to commercial brands that have praziquantel and fenbendazole as active ingredients. Another alternated between anthelmintics from different chemical classes, involving a rotation of compounds containing febantel, pyrantel, and praziquantel, a praziquantel-only

product, and a compound containing milbemycin oxime (macrocyclic lactone) and praziquantel.

The study design focused on recording whether sampled animals had received AH treatment within the three months before sampling, rather than collecting data on specific deworming intervals or post-treatment faecal examination practices. This approach was chosen to align with the study's primary objectives and to complement our research on shelter deworming practices in the country (Lopes et al., 2022).

Ten shelters provided data on animals' mean length of stay (LOS), indicating an average stay of 1–2 years. Among the 19 surveyed shelters, six did not allow animals to walk outside the shelter facilities. Only two shelters permitted occasional walks, while two others allowed weekly walks. Information regarding outdoor access for the remaining nine shelters was not obtainable. Typically, with some exceptions, official shelters in Portugal are situated in peripheral areas of urban centres with green spaces nearby. This is the case for the kennels that participated in this study, particularly those that reported animals taking leashed walks outside with volunteers. These walks generally occur in these nearby green areas.

2.3. Individual and pooled group faecal samples

A total of 355 faecal samples were examined, comprising 88 from the North, 95 from the Centre, 20 from the Lisbon Metropolitan Area, 82 from the Alentejo, 30 from the Algarve, and 40 from the island of S. Miguel, Autonomous Region of Azores (Fig. 1). Table 1 summarises the metadata of samples from the study. Most samples were collected during autumn, with a smaller proportion collected in spring. The samples were predominantly obtained from adult male dogs captured by shelter staff from the streets. The proportion of samples from dogs dewormed over three months ago (54.9 %) did not markedly differ from those dewormed less than three months ago (43.7 %). Additionally, 42.3 % of samples exhibited an unformed consistency (Table 1). This consistency alteration was particularly significant in juvenile and adult dogs up to seven years of age ($p < 0.001$).

2.4. Copro-parasitological examination

To ensure biosafety, copro-parasitological examinations were performed in a securely enclosed environment, with the use of protective clothing, as there was a risk that faecal samples could contain *Echinococcus granulosus*.

The copro-parasitological examination involved an initial macroscopic examination of the samples to detect any signs of disease or parasitic infection. Faecal consistency (i.e., formed or unformed), presence of blood or mucous, and adult parasites or cestode proglottids were documented for each sample. Subsequently, all samples were analysed using the Mini-FLOTAC technique. Briefly, 2 g of faeces were added to a Fill-FLOTAC device and homogenised with 38 mL of zinc sulphate flotation solution (specific gravity 1.35), prepared as previously described (Cringoli et al., 2017), to achieve a dilution ratio of 1:20. The samples were then examined under a light microscope at total magnifications ranging from 100× to 400×. Parasitic forms were identified through their morphological and morphometric characteristics based on established references (e.g., Soulsby, 1982; Sloss et al., 1994; Thienpont et al., 2003; Bowman, 2020) and quantified. The total number of parasitic forms counted in the two chambers of the Mini-FLOTAC device was multiplied by a factor of 10 to obtain the final count in eggs or oocysts per gram of faeces (EPG and OPG, respectively). The Mini-FLOTAC technique enabled the determination of shedding intensity for each GIP in terms of EPG or OPG.

A dog or group of dogs were classified as positive if at least one parasitic form (i.e., eggs, cysts, oocysts, and larvae of parasites) was detected in their faecal samples. The overall prevalence was calculated as the percentage of faecal samples positive for parasites. Specific prevalence was calculated as the percentage of faecal samples positive

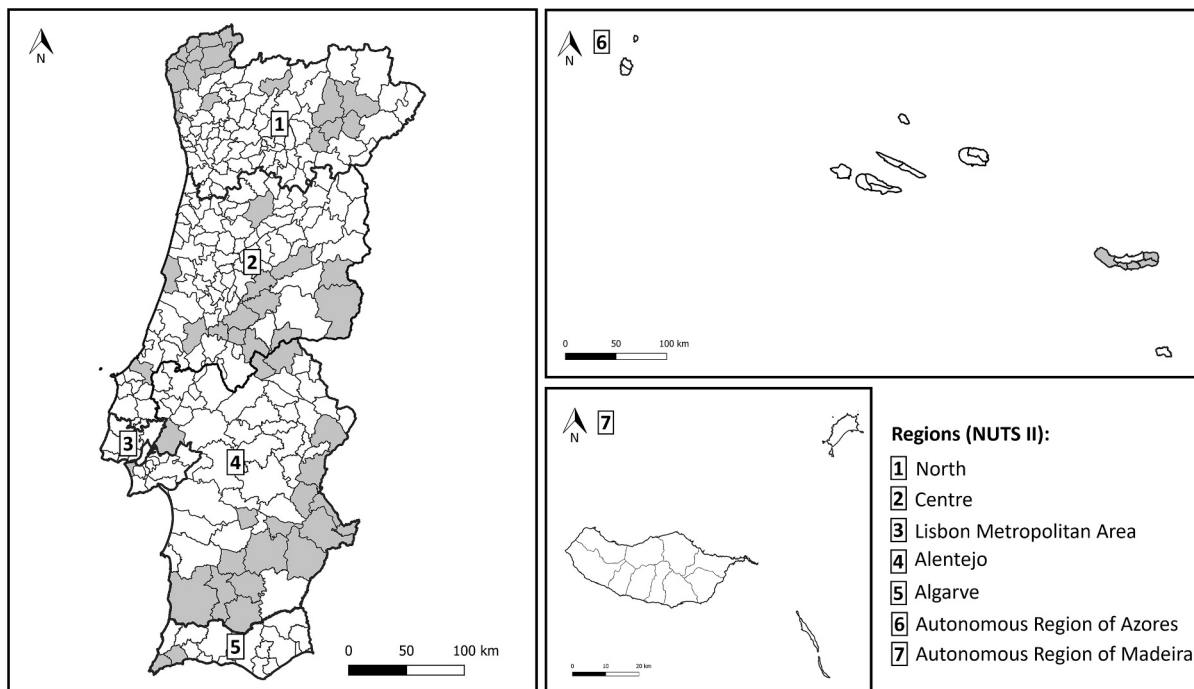


Fig. 1. Representation of six out of the seven NUTS II regions included in the study. The territorial area of Portugal covered by the participating municipal shelters is filled in grey. Original map created in QGIS 3.22.14 Białowieża.

Table 1
Metadata of analysed faecal samples (n = 355).

Variable	Number of samples (%)
Sampling season	
Fall	184 (51.8 %)
Winter	72 (20.3 %)
Spring	7 (2.0 %)
Summer	92 (25.9 %)
Sample type	
Individual	208 (58.6%)
Pooled Group	147 (41.4 %)
Origin of the animal(s)	
Stray animal(s) of national origin captured	214 (60.3 %)
Dog(s) surrendered by owners and non-owners	76 (21.4 %)
Animal impounded by official authorities	51 (14.4 %)
Various origins in an animal group*	14 (3.9 %)
Sex of dogs	
Female	106 (29.9 %)
Male	174 (49.0 %)
Group of males and females	75 (21.1 %)
Dogs' age range	
Juvenile (≤ 12 months)	23 (6.4 %)
Juveniles with adults	8 (2.2 %)
Adults (> 12 months to <7 years)	273 (76.9 %)
Adults with geriatrics	9 (2.5 %)
Geriatric (≥ 7 years)	42 (11.8 %)
Most recent anthelmintic treatment before sampling	
Less than 3 months	155 (43.7 %)
Last 3 days	5 (1.4 %)
Over 3 months	195 (54.9 %)
Faecal consistency	
Formed	205 (57.7 %)
Unformed	150 (42.3 %)

* Various situations in an animal group: This category refers to group samples containing animals with mixed origins, such as a combination of stray animals, dogs surrendered by owners and non-owners, and animals impounded by official authorities.

for a specific parasite species.

Unlike production animals, in which shedding intensity is often classified as low, medium, or high, based on Soulsby's (1982)

classification, there is currently no universally accepted shedding intensity classification for companion animals. Consequently, the frequency of parasitic forms in each faecal sample (i.e. shedding intensity) was determined to practically estimate the environmental contamination potential of the infected animal, given the absence of consensus classification criteria. Subsequently, the parasite shedding intensity associated with each form was categorised as low (n = 1–9 eggs or oocysts), medium (10–50), or high (>50).

For samples harbouring Taeniidae eggs, an additional faecal flotation with centrifugation was performed to ascertain the presence of *E. granulosus*. This technique aimed to isolate and concentrate the parasite eggs for molecular testing. The methodology employed for this purpose was adapted from Maurelli et al. (2018) and is elucidated in Supplementary Fig. 1.

2.5. Molecular identification of taeniid egg isolates

Prior to DNA extraction from the isolated Taeniidae eggs, a thermal treatment was performed to facilitate the chemical-enzymatic degradation of the egg shell.

The thermal shock protocol, adapted from Hidalgo et al. (2018), facilitates DNA extraction without compromising DNA integrity. Initially, the isolated Taeniidae egg samples stored at –20 °C underwent incubation at 65 °C for 20 min. Subsequently, the samples were subjected to four cycles of freezing at –20 °C (each lasting ten minutes) interspersed with four cycles at 65 °C (each lasting three minutes). Samples were then promptly cooled on ice for five minutes. Finally, the QIAamp PowerFecal Pro DNA Kit (Qiagen, GmbH, Hilden, Germany) was utilised to extract total DNA according to the manufacturer's instructions.

The presence of *E. granulosus* DNA in faecal samples was assessed using multiplex Polymerase Chain Reaction (PCR) as described by Trachsel et al. (2007). This multiplex PCR was developed for identification of taeniid eggs of the species *E. granulosus*, *Echinococcus multilocularis* or *Taenia* species. PCR aimed to amplify targets within the mitochondrial NADH dehydrogenase subunit 1 gene (*nad1*) for *E. multilocularis*, and the small subunit of ribosomal RNA (*rrnS*) for

E. granulosus and *Taenia* species, employing the specific primers Cest1-Cest5 (Trachsel et al., 2007). All PCRs were performed in a Biometra P Cycler (Biometra Inc., Göttingen, Germany) in a 50 µL volume reaction containing 25 µL of master mix 2× (NZYTaQ II 2× Green Master Mix, NZYTech, Portugal), 2 µL of template DNA, 5 µL of primer mix (2 mM of primers Cest1, Cest2, Cest3, Cest4 and 16 mM of primer Cest5 in Tris-EDTA or H₂O), and 18 µL H₂O. Thermocycling parameters included an initial step at 95 °C for 15 min, followed by 40 cycles at 94 °C for 30 s, 58 °C for 90 s, and 72 °C for 25 s, with a final elongation step at 72 °C for 10 min. Subsequent analysis involved electrophoresis on a 1.5 % agarose gel (w/v) stained with GreenSafe Premium DNA gel stain (NZYTech, Portugal). PCR products, including the DNA from hydatid cysts of *E. granulosus* (used as positive controls), were purified, and commercially sequenced using the Sanger method at Eurofins Genomics (Germany). All obtained sequences were compared to GenBank® sequences using the National Center for Biotechnology Information (NCBI)'s Basic Local Alignment Search (BLAST) online platform.

2.6. Statistical analysis

Data analysis was conducted using R software (Version 4.2.2). Fisher's exact tests were employed to evaluate the correlations between sample metadata (i.e., animal- and shelter-related variables) and the occurrence (presence/absence) of GIP. Specifically, the influence of region, number of dogs housed, pest presence, origin of the animal, last AH treatment, season, sex, age, and faecal consistency on the occurrence of GIP was assessed, with a *p*-value <0.05 considered statistically significant. Exact binomial 95 % confidence intervals (CI) for proportions were computed.

3. Results

3.1. Occurrence, diversity and shedding intensity of GIP

Among the 355 dog faecal samples examined (complete dataset, including group and individual samples), 16.1 % (95 % CI 12.4–20.3 %) tested positive for at least one GIP. Morphometric data supporting taxonomic assignments of parasitic forms observed are provided in the caption of Supplementary Fig. 2. Six faecal samples exhibited two parasite taxa in the same sample. Two individual dog samples contained *Cystoisospora canis* and *T. vulpis*. One pooled group sample contained *T. canis* and *Toxascaris leonina*, while the remaining three samples, one individual sample and the other two from two pooled groups of animals, contained *T. canis* and *T. vulpis*. The Centre region and the island of S. Miguel, Autonomous Region of Azores, had the highest prevalence of

GIP-positive samples, with rates of 23.2 % (95 % CI 15.1–32.9 %) and 32.5 % (95 % CI 18.6–49.1 %), respectively (Fig. 2A and Supplementary Table 2). Overall, *T. vulpis* (5.6 %, 20/355), *T. canis* (5.4 %, 19/355), *Ancylostoma* spp. (3.7 %, 13/355), and *C. canis* (2.3 %, 8/355) were the most common GIP. The Alentejo region exhibited the highest GIP diversity, with five out of the six taxonomic groups detected in this study (Fig. 2). The single taeniid-positive sample detected was from this region. Specifically, a case of *E. granulosus* sensu lato (s.l.) was identified and confirmed through PCR and sequencing, representing a prevalence of 1.2 % (95 % CI 0.03–6.6 %) in the region and 0.3 % (95 % CI 0.0–1.6 %) in the country. Additionally, the case of co-recovery of *T. canis* and *T. leonina* in a pooled group sample was also observed in the Alentejo region, as well as a case involving eggs of the Anoplocephalidae family tapeworm, a spurious parasite not known to infect dogs, was also observed in the Alentejo region, likely resulting from the ingestion of contaminated food or even ruminant faeces. The infection patterns in the North and Algarve regions mirrored those observed across the complete dataset, with *T. vulpis* and the *T. canis* being the most prevalent GIP. Conversely, *Ancylostoma* spp. were the predominant GIP found in animals from the Alentejo region and the island of S. Miguel, Autonomous Region of Azores (Fig. 2A and Supplementary Table 2).

Focusing the analysis on data from individual samples only (*n* = 208), the overall prevalence of GIP-positive samples was 15.9 % (95 % CI 11.2–21.6 %). Regarding specific parasite prevalence, *T. vulpis* and *T. canis* both exhibited a positivity rate of 5.3 % (11/208), *C. canis* 3.4 % (7/208), *Ancylostoma* spp. 2.4 % (5/208), while *Dipylidium caninum* and *E. granulosus* (s.l.) both showed a positivity rate of 0.5 % (1/208). The percentage of samples positive for GIP remained highest in the Central region and in the island of S. Miguel, Autonomous Region of Azores, with rates of 25.0 % (95 % CI 14.0–38.9 %) and 28.6 % (95 % CI 3.7–57.9 %), respectively (Fig. 2B and Supplementary Table 3). However, considering individual samples only, the Alentejo region exhibited a GIP positivity rate closer to these two regions, with an overall GIP-positive sample percentage of 20.0 % (95 % CI 9.1–35.6 %). Nevertheless, the Alentejo region still presented the most diverse GIP panel (Fig. 2B and Supplementary Fig. 2).

T. canis (11.5 %) was the most prevalent GIP in the Centre region, followed by *T. vulpis* and *C. canis* species, each representing 9.6 %. On the island of S. Miguel, Autonomous Region of Azores, *T. vulpis* and *Ancylostoma* spp. were the most prevalent GIP, both with a frequency of 14.3 %. No considerable differences in GIP shedding frequency were denoted in the remaining regions when considering individual samples exclusively (Fig. 2B and Supplementary Table 3).

The analysis of parasite diversity and prevalence by age group revealed that among juveniles *T. canis* was the only parasite detected in

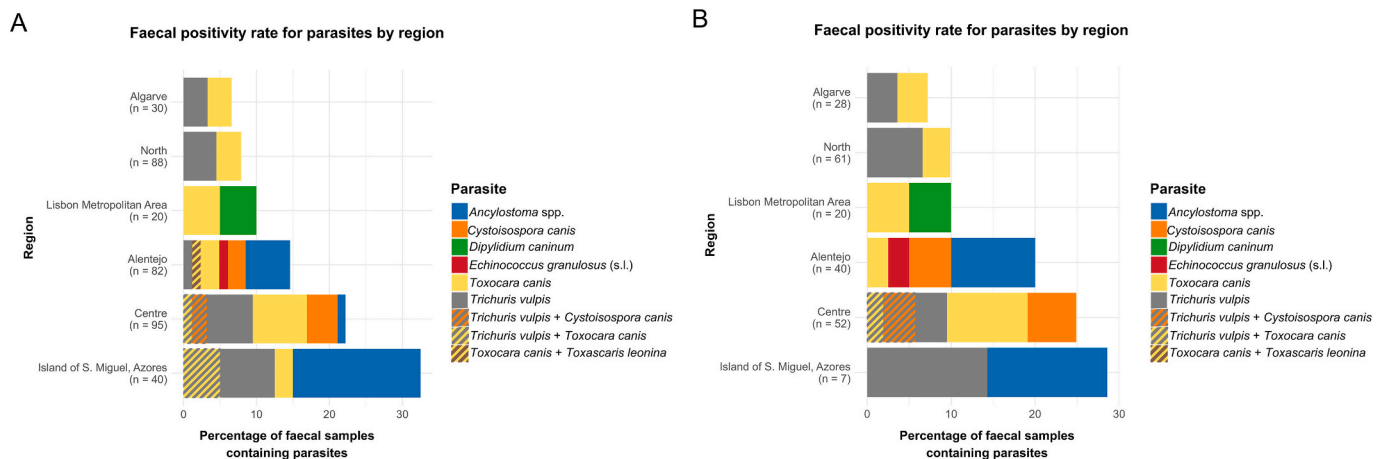


Fig. 2. Regional distribution of canine parasitic infections. A) Percentage of GIP-positive among the total 355 faecal samples analysed from six NUTS II regions, Portugal. B) Prevalence of GIP in individual faecal samples (*n* = 208) across six NUTS II regions, Portugal.

faecal samples. In contrast, adult animals exhibited a broader spectrum of parasitic fauna, with *T. canis* also present in this group. Geriatric animals showed the highest prevalence of *C. canis* infection. Additionally, the single case of taeniid infection was identified in the geriatric age group (Fig. 3 and Supplementary Table 4).

The mean shedding intensity and range of eggs per gram of faeces (EPG) for individual animals that were positive for *Ancylostoma* spp., *T. canis*, and *T. vulpis* are depicted in Table 2. Based on the intensity criterion, the mean shedding intensity found for those GIP in individual dogs is considered high (above 50 EPG). Moreover, the sample with 10 EPG of *E. granulosus* (s.l.) represented a shedding rate at the lower-middle limit.

T. canis eggs were present in 13.0 % of juveniles (three out of 23, 95 % CI 2.8–33.6 %) and 5.1 % of adults (14 out of 273, 95 % CI 2.8–8.5 %) in both individual and group samples of the same age group. Samples from groups of juveniles housed in the same enclosure with adults revealed an infection rate of 25 % (two out of eight, 95 % CI 3.2–65.1 %). No *T. canis* eggs were detected in samples obtained from geriatric animals (individual or housed in groups).

3.2. Factors influencing GIP occurrence

Significant associations were found between sample metadata variables and occurrence (presence/absence) of GIP. Specifically, the higher number of animals housed in shelters was significantly related to a higher number of positive *Ancylostoma* spp. ($p = 0.009$) and *T. vulpis* ($p = 0.03$) cases. *Ancylostoma* spp. egg shedding was significantly higher in the Alentejo region and on the island of S. Miguel, Autonomous Region of Azores ($p < 0.001$), and during autumn and summer ($p = 0.002$). Furthermore, higher probability of *T. canis* infection and GIP co-infection were significantly associated with unformed faeces ($p = 0.006$ and $p = 0.02$, respectively). An association close to statistical significance was also observed between age group and the presence of *T. canis* eggs ($p = 0.053$). Additionally, the frequency of walks taken outside the shelter facilities was marginally significantly associated with the presence of parasites, indicating that animals without walks, with occasional walks, or with weekly walks may have differing probabilities of being infected with GIP ($p = 0.049$). The percentage of positive samples was slightly higher in shelters that permitted occasional walks (23.8 %) compared to those that did not permit dog walks outside the shelter facilities (19 %). Interestingly, a shelter housing 42 dogs, which allowed weekly outdoor activities, reported a 10 % rate of GIP-positive cases.

A significant association was also observed between the presence of

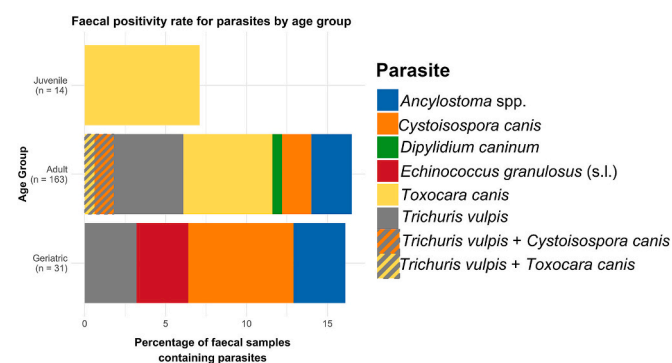


Fig. 3. Diversity and relative frequency of gastrointestinal parasites (GIP) in individual samples ($n = 208$), stratified by age groups. Each bar represents an age group, with its width proportional to the percentage of GIP-positive samples within that group. Coloured sections within each bar indicate different parasite taxonomic groups, with section size reflecting the relative frequency of each parasite within the age group. The total sample size (n) for each age group is provided.

Table 2

Mean shedding rate with standard error of the mean (SEM) and range of eggs per gram of faeces (EPG) for positive individual samples with *Ancylostoma* spp., *Toxocara canis* and *Trichuris vulpis*.

GIP	Number of positive individual samples	Mean intensity (EPG)	Standard Error of the Mean (SEM)	Range (EPG)
<i>Ancylostoma</i> spp.	5	278.0	74.5	110–600
<i>Toxocara canis</i>	10	135.5	39.6	10–370
<i>Trichuris vulpis</i>	11	87.3	52.7	10–610

parasitism and the origin of the animals ($p = 0.022$). Animals impounded by authorities exhibited the highest GIP-positive rate (29.4 %), followed by group samples comprising animals of mixed origins (28.6 %). Among captured stray animals of national origin, 14 % of faecal samples were positive for parasites. The lowest prevalence was observed in animals surrendered by owners and non-owners (10.5 % testing positive).

No correlations were observed between samples from dogs that were dewormed within three months, or less than three months ago, and infection with specific GIP taxonomic groups. Specifically, the presence of *Ancylostoma* spp. ($p = 0.2449$), *T. canis* ($p = 0.2974$), and *T. vulpis* ($p > 0.999$) showed no significant correlation with the timing of the last deworming treatment. Similarly, no significant correlations were found between sex and the presence of *Ancylostoma* spp. ($p = 0.3173$), *T. canis* ($p = 0.4653$), or *T. vulpis* ($p = 0.1964$). Additionally, the presence or absence of pests did not significantly correlate with the presence of *Ancylostoma* spp. ($p = 0.6262$), *T. canis* ($p > 0.999$), or *T. vulpis* ($p = 0.4294$).

No significant association was observed between the LOS at the shelters and the occurrence of GIP (presence/absence) ($p = 0.754$). The average rate of GIP-positive samples for dogs with a LOS under one year was 20.9 %, while for those housed between 1 and 2 years, it was 18.1 %. Lastly, for those with LOS between 2 and 3 years and between 3 and 4 years, the average rate was 12.5 %. However, it should be noted that this analysis was conducted with a considerably small sample size, as only ten out of the 19 participating shelters were able to provide this specific information. The limited number of observations may have impacted the ability to detect any significant association.

4. Discussion

Shelter dogs are significantly more prone to parasitic infections due to the high and persistent transmission pressure within these populations (Scaramozzino et al., 2018). Their living conditions render them especially vulnerable to GIP, increasing the risk of environmental contamination and serving as sources of infection for arthropod vectors, other animals, and humans (Traversa et al., 2017). While there have been regional studies on GIP occurrence in stray and shelter dogs in various parts of Portugal, these studies are limited to individual shelters or restricted areas, leading to a fragmented understanding of the issue. This study addresses this significant gap by presenting the first nationwide survey of GIP in shelter dogs across Portugal, revealing an overall prevalence of parasitism of 15.9 %. This prevalence is notably lower than previously reported rates in Portuguese shelter dogs, such as the 69.6 % documented by Martins et al. (2005) and the 57.2 % reported by Silva et al. (2020). A recent study conducted in the municipality of Viseu, in the north-central region of Portugal, by Rocha (2022) found that none of the animals housed in a shelter were parasitised post-admission, whereas 62.5 % (15 out of 24) of stray dogs were parasitised before receiving deworming treatments. Additionally, the parasitism rate among owned dogs living in rural or semi-urban

environments was 29.4 % (5 out of 17). These findings and the lower parasitism rate observed in our study underscore the effectiveness of implementing deworming protocols upon each dog's shelter admission. These findings are also consistent with the results reported by Lebre (2011), who noted a parasitism rate of 23.4 % in a similar context to that of our study and Rocha's study (Rocha, 2022). Copro-parasitological surveys in dogs from other regions of the world report a wide range of estimated overall prevalence (9.4 % to 85.0 %) (Supplementary Table 1) and reveal marked variations in parasite species diversity and in the identity of the predominant GIP (Mateus et al., 2014).

The diversity of results in GIP studies can be attributed to several factors, including environmental conditions such as soil type, elevation, geographical location, and climate (Robertson et al., 2000; Mateus et al., 2014; Ferreira et al., 2017; Liberato et al., 2018); dog population (e.g., household, shelter, stray, farm or hunting dogs) (Robertson et al., 2000; Ferreira et al., 2011; Crespo et al., 2006; Mateus et al., 2014; Matos et al., 2015; Liberato et al., 2018; Scaramozzino et al., 2018); sampling period (e.g., warmer months are not suitable for the protozoa life cycle) (Crespo et al., 2006); collection method (e.g., collection of samples per rectum, from the ground or in necropsy) (Robertson et al., 2000; Mateus et al., 2014); prior AH usage (Robertson et al., 2000; Matos et al., 2015; Ferreira et al., 2017; Liberato et al., 2018; Scaramozzino et al., 2018); and coprological techniques employed (Robertson et al., 2000; Mateus et al., 2014). This variability underscores the importance of conducting research in different contexts to improve our understanding of GIP bioecology, particularly for zoonotic parasites. Animal management factors, particularly the level of veterinary care and attention from caretakers, also play a crucial role (Matos et al., 2015). Our findings corroborate this, revealing a significant association between animal origin and parasitism prevalence. Officially impounded animals exhibited the highest rate of parasitism (29.4 %), whilst those surrendered by owners showed the lowest (10.5 %). This difference suggests that dogs with previous owners likely received regular veterinary care, and animals in shelters with effective protocols may have lower parasitism rates. These findings highlight the importance of consistent preventive care and well-implemented shelter management strategies in reducing GIP infections.

Human-induced changes in landscapes, such as encroachment on natural habitats, increased pet tourism and international pet adoption, and the movement of exotic species (e.g. raccoons) through legal or illegal channels, can affect the prevalence of intestinal parasites. Such modifications promote more opportunities for interaction between domestic and wild carnivores, facilitate the spread of infected hosts into new areas and may introduce novel parasite species and associated diseases (Otranto et al., 2015). In addition, the extent of interaction dogs have with other canid species also has a substantial impact, as stray and feral dogs will have more contact with wild canids, as will dogs in rural environments than pet dogs in urban settings (Cleveland et al., 2001; Craft et al., 2017; Sanchez-Thevenet et al., 2019). Given this variability, it is essential to exercise caution when extrapolating conclusions from one location to another. This highlights the necessity of conducting comprehensive studies in different demographic regions, such as the present study, to gain a deeper understanding of GIP epidemiology.

Our findings indicate a higher prevalence of helminth eggs than protozoa, consistent with prior research on shelter dogs (Liberato et al., 2018; Scaramozzino et al., 2018). However, it is essential to acknowledge the limitations of our diagnostic approach. Despite the fact that the Mini-FLOTAC technique uses a zinc sulphate solution (specific gravity 1.35) offering relatively high sensitivity for detecting and quantifying helminth and protozoa stages shed in faeces, its sensitivity for protozoan detection may be lower than other methods and when using other flotation solutions (e.g., saturated glucose-salt solution). Moreover, the specific gravity of the flotation solution significantly influences the number and types of parasites recovered, even when using the same flotation solution (Cringoli et al., 2017).

The absence of *Giardia duodenalis* and *Cryptosporidium canis* in our

results merits discussion. Reported prevalence rates of *G. duodenalis* in companion animals vary considerably across studies, largely influenced by differences in diagnostic sensitivity and the number of samples examined due to intermittent cyst excretion (Ferreira et al., 2011). Similarly, the detection of *C. canis* is also dependent on the diagnostic technique employed. By relying solely on Mini-FLOTAC and not employing additional or alternative methods (e.g., formalin-ether concentration method (FECM), antigen detection assays, or PCR assays, among others), we may have underestimated the actual prevalence of these and other protozoan parasites (Neves et al., 2014; Cringoli et al., 2017). Additionally, the age distribution of our sample population might have influenced our results, as certain protozoan infections are less common in older animals (Vrhovec et al., 2022). Of the 355 samples analysed, 6.4 % (23/355) were from juveniles alone, 76.9 % (273/355) from adults alone, and 11.8 % (42/355) from geriatric dogs alone. The remaining samples came from mixed age groups (2.2 % juveniles with adults and 2.5 % adults with geriatrics). Some studies indicate that parasite fauna between stray and household dogs may differ (Matos et al., 2015; Scaramozzino et al., 2018). Increased owner awareness of preventive AH treatments has reduced intestinal helminths in household dogs (Mateus et al., 2014; Ferreira et al., 2017). Simultaneously, a higher protozoal infection trend has been observed (Mateus et al., 2014; Ferreira et al., 2017). A possible explanation is that despite dewormers such as febantel and fenbendazole having potential efficacy against *G. duodenalis*, the standard preventive AH protocols are not designed to target these infections (Ferreira et al., 2017). Consequently, household dogs may still harbour protozoan infections despite regular AH treatments. Conversely, stray animals may have a higher helminth prevalence because they typically do not receive any deworming treatment (Mateus et al., 2014).

Regional variations in parasite prevalence have been observed in Portugal, with some districts or regions showing no protozoan infections and others finding them most frequent (Félix, 2015; Ferreira et al., 2023). These disparities could be attributed to diagnostic technique, geographical variations, environmental factors, and differences in shelter management practices, such as sanitisation and disinfection protocols and AH treatments. Future studies should consider employing multiple diagnostic techniques, including antigen tests and PCR assays, to provide a more comprehensive picture of GIP prevalence, especially for protozoan parasites like *G. duodenalis* and *C. canis*.

Consistent with previous research in specific geographic regions of Portugal, *T. vulpis*, *T. canis*, and *Ancylostoma* spp. were identified as the most prevalent GIP in dogs (Crespo and Jorge, 2000; Martins et al., 2005; Crespo et al., 2006; Cardoso et al., 2014; Mateus et al., 2014). However, earlier studies predominantly detected Ancylostomatidae (hookworms) as the most prevalent (Crespo and Jorge, 2000; Martins et al., 2005; Crespo et al., 2006; Cardoso et al., 2014; Mateus et al., 2014; Ferreira et al., 2017; Silva et al., 2020; Idrissi et al., 2022). Ferreira et al. (2017) found hookworms to be the most prevalent GIP in various dog populations across Portugal.

This study, however, revealed significant regional variations in the most frequent GIP. *Ancylostoma* spp. occurrence was notably higher in the Alentejo region and the island of S. Miguel, Autonomous Region of Azores. According to Bowman (2020), soil characteristics significantly influence hookworm development, with gravel and sand being ideal substrates and hookworm larvae developing well in shaded areas of well-drained soils but not in heavy, waterlogged soils. Additionally, optimal development occurs between 23 °C and 30 °C in moderately moist, well-aerated mediums. The Alentejo region generally experiences hot and dry summer conditions and is characterised by diverse soil compositions, including clay-rich soils in many areas that might seem challenging for hookworm larvae survival (IPMA, 2025). However, specific microclimate conditions and localized soil compositions may create favourable niches for their development, warranting further investigation. Conversely, the temperate climate and high humidity of the island of S. Miguel, Autonomous Region of Azores create conditions

that likely support the survival of hookworm larvae, explaining the higher prevalence observed in this region. In another study examining GIP in samples from shelter and household dogs on the islands of S. Miguel and Terceira, Autonomous Region of Azores, similar to our findings for the S. Miguel, hookworms parasites were the most frequently found (Teixeira, 2020).

Palmer et al. (2007, 2008) and Nath et al. (2023) emphasised climate's role in hookworm distribution and prevalence, aligning with our findings. Illiano et al. (2023) suggested that climatic changes and increased animal movements due to globalisation might explain *Uncinaria stenocephala* occurrences in suboptimal climates. These findings highlight the intricate relationship between environmental factors and hookworms prevalence, underscoring the need to consider environmental dynamics in parasite epidemiology. Although our protocols for handling samples were intended to minimize egg degradation, the lower detection of hookworms eggs may also be due to their inherently lower resistance to environmental factors compared to the eggs of *T. canis* and *T. vulpis* (Cardoso et al., 2014).

In this study, the average LOS in the shelter ranged from one to two years. Marques et al. (2023) defined LOS at shelters as excessive when over half of the animals stay for more than a year. According to this definition, slightly more than half of the participating shelters (10 out of 19) could be categorised as high LOS kennels. In our study, GIP-positive cases were higher in dogs with a LOS under one year (20.9 %) and decreased with longer LOS, suggesting that extended LOS might offer protection against GIP due to established deworming protocols. However, no statistically significant association was found between LOS and the occurrence of parasitism. It should be noted, however, that this analysis was conducted with a small sample size.

Our previous study (Lopes et al., 2022) obtained responses from 61 % (83/136) of veterinarians working in official shelters across the country, including the islands, providing a comprehensive national perspective. This previous study reported that 95.2 % of municipal veterinarians reported deworming dogs upon shelter entry, and 95.5 % had an AH protocol in place. Our current study found that all participating shelters (19/19) implemented deworming protocols. These established practices likely contribute to the lower overall GIP prevalence (15.9 %) observed in our current study compared to previous national studies.

Despite the widespread implementation of AH protocols, our previous research (Lopes et al., 2022) uncovered significant gaps in parasite control practices. Many shelters neglected to properly collect and dispose of faeces after deworming, especially during the critical three-day post-treatment period. This oversight can compromise deworming efficacy and needs addressing through enhanced staff and volunteer training in sanitation and infection control practices.

Furthermore, our prior study revealed a lack of routine copro-parasitological analyses in nearly all participating shelters. Echoing the recommendations of Traversa (2012), we strongly advocate conducting periodic copro-parasitological examinations, as they enable targeted treatment of infected animals, prevent unnecessary drug administration, and are essential for evaluating the success of the administered AH and for detecting early signs of resistance (Traversa, 2012).

Outdoor activity external to shelter facilities appears to influence the prevalence of GIP. Shelters permitting occasional walks exhibited a modestly higher GIP prevalence (23.8 %) than those with no outdoor access (19 %) outside the shelter facilities. However, a shelter with weekly walks had a significantly lower prevalence (10 %). These findings suggest that although LOS and outdoor activities beyond the shelter premises may influence parasite prevalence, other management and maintenance practices, such as proper hygiene and animal density management, are also critical. Infection risks are more manageable in shelters with fewer animals, such as the one allowing weekly walks with 42 animals housed.

The peripheral urban location of shelters near green spaces

introduces additional variables in parasite exposure. While leashed walks with volunteers provide environmental enrichment, they may not prevent all risk behaviours. Dogs might still graze on grass, potentially ingesting parasites (Traversa et al., 2014). Although volunteers are typically equipped with waste collection bags, the consistency of faecal collection and disposal practices may vary, potentially influencing environmental contamination and subsequent parasite exposure risks. The lower prevalence in the shelter with weekly walks could be attributed to more structured protocols or better-trained volunteers, possibly including more consistent faecal management practices.

Future research should investigate the complex interplay between walk frequency, environmental characteristics, behavioural control during walks, and volunteer hygiene practices. Such studies could optimise outdoor enrichment while minimizing parasite transmission risks in shelter dog populations.

The combination of AH drugs praziquantel-pyrantel with an additional molecule (either febantel or fenbendazole) was the most frequently reported AH protocol. Previous studies identified these combinations as the most used in shelters and privately owned dogs (Lopes et al., 2022; Matos et al., 2015). Judicious use of AH drugs is paramount, given the established evidence of parasite resistance to these medications in small animals. Multiple drug resistance (MDR) to benzimidazoles, macrocyclic lactones, and pyrantel in *Ancylostoma caninum* has been conclusively demonstrated in multiple isolates from dogs in the United States, with evidence suggesting this is an emerging problem (Castro et al., 2019). Additionally, recent reports have identified cases of praziquantel resistance in *Dipylidium caninum*, including documented cases in Europe (Oehm et al., 2024). Resistance to macrocyclic lactones in *Dirofilaria immitis* has also been well documented (Bowman, 2012).

The emergence of AH resistance in European canine populations is now an established concern, though its full extent remains largely unexplored. Careful administration of these treatments is essential to maintain their efficacy and protect animal health in the long term. This highlights the urgent need for systematic post-treatment efficacy assessments in clinical practice and research studies to monitor resistance patterns and inform evidence-based treatment protocols. Given that resistance may be more widespread than previously recognised, parasitological surveillance with appropriate diagnostic methods should be implemented to guide treatment decisions and detect emerging resistance.

Our previous research (Lopes et al., 2022) also highlighted that the most common frequency of deworming in municipal kennels was every six months, mirroring findings from studies of privately owned dogs (Ferreira, 2015; Nabais, 2008, as cited in Ferreira, 2015; Matos, 2013, as cited in Ferreira, 2015). Without routine coprological analyses, this frequency may be insufficient, potentially leaving animals vulnerable to parasitic infections. Considering these findings, we recommend that shelters adopt a more comprehensive approach to parasite control. This should include regular copro-parasitological screening, targeted treatment based on diagnostic results, proper post-treatment faecal management, and ongoing monitoring for signs of drug resistance. By implementing these measures, shelters can enhance the efficacy of their parasite control programmes while promoting responsible AH use.

Most shelters (63.2 %) in this study reported a recent surge in incoming dogs. Our previous study (Lopes et al., 2022) found that 85.1 % of official shelters lack sufficient space to house dogs properly, suggesting overcrowding issues. Additionally, three shelters in the current study that were not experiencing increased arrivals indicated that their shelters had already exceeded capacity.

These findings underscore the critical need for enhanced staff training on proper faeces removal and disposal practices post-deworming. Our previous research highlighted deficiencies in sanitation and infection control training, particularly regarding handling faeces potentially contaminated with parasites (Lopes et al., 2022). Many shelters fail to follow appropriate faeces disposal practices and lack adequate isolation areas for newly arrived animals (Lopes et al.,

2022).

Quarantine, recommended for at least seven to ten days (Morris, 2018; Raza et al., 2018), is essential for preventing disease transmission. This measure, effective AH treatment, and environmental management are crucial for reducing dogs' risk of GIP infections. Proper ecological management includes the implementation of rigorous sanitation (i.e., mechanical cleaning to physically remove as many parasite forms as possible from the environment, regular disinfection and cleaning of living areas, appropriate and rapid faeces disposal), adequate housing, and reduced overcrowding (Raza et al., 2018).

Previous studies have consistently reported a higher prevalence of single infections compared to mixed infections (Ramirez-Barríos et al., 2004; Fontanarrosa et al., 2006; Ugbomoiko and Heukelbach, 2008; Bwalya et al., 2011; Ferreira et al., 2011; Okoye et al., 2011; Neves et al., 2014; Ferreira et al., 2023). The interaction dynamics between different parasite species may be primarily influenced by the parasite load rather than the mere coexistence of other species (Fontanarrosa et al., 2006). Schmidt and Roberts (2009) further suggested that the coexistence of multiple parasites within a single host only occurs if they are not in competition or have divergent physiological needs.

The prevalence of *Ancylostoma* spp. and *T. vulpis* was significantly higher in shelters with larger canine populations. This aligns with Scaramozzino et al. (2018) findings, which reported a higher prevalence of these GIP in shelter dogs compared to household dogs. This trend can be attributed to similar living conditions conducive to the proliferation of immature stages of these GIP, particularly prevalent in confined spaces such as animal shelters. Additionally, severe *T. vulpis* infections have been predominantly observed in shelter environments (Ferreira et al., 2017).

Earlier studies did not establish an association between *T. canis* infection or mixed infections and unformed faeces. However, the current study identified a significant association. Numerous studies have reported a significantly higher prevalence of *T. canis* infection in puppies, primarily driven by vertical transmission pathways, including transplacental and transmammary routes, which are the predominant modes of infection in newborn puppies (Idrissi et al., 2022; Csokai et al., 2024). Additional factors contributing to infection include immunological immaturity and the stress from weaning, transportation, and re-homing (Neves et al., 2014). Similarly, shelter animals are more susceptible to stress, which may lead to gastrointestinal distress and subsequently result in altered faecal consistency (Liberato et al., 2018). Furthermore, in the present study, changes in faecal consistency were more prevalent in young and adult dogs up to seven years old ($p < 0.001$). An association approaching statistical significance was found between age and *T. canis* infection ($p = 0.053$), with a higher prevalence in younger animals and no cases detected in geriatric dogs (above 7 years old). This trend aligns with previous studies reporting age-related patterns of *T. canis* infections (Neves et al., 2014; Idrissi et al., 2022; Csokai et al., 2024).

Contrary to prior research, this study found no correlation between age and *T. vulpis* infection (Liberato et al., 2018). Previous studies suggested that the lack of regular AH treatments and the high environmental resistance of *T. vulpis* eggs could cumulatively impact infection rates over a dog's lifespan (Liberato et al., 2018).

Preceding studies have documented a significantly higher prevalence of *Cystoisospora* spp. infection in younger dogs than adults (Martínez-Moreno et al., 2007; Becker et al., 2012; Garcia-Campos et al., 2018). Specifically, research has shown that puppies under six months old have the highest infection rates, possibly due to their immature immune systems (Fontanarrosa et al., 2006; Martínez-Moreno et al., 2007; Idrissi et al., 2022). However, the current study found no *C. canis* infections in juvenile dogs, with the highest incidence observed in geriatric animals. A survey conducted in the USA on the most common canine and feline endoparasites in thousands of pets revealed that, after animals under 6 months of age (as anticipated), the most parasitised category of animals is those over 10 years old (Gates and Nolan, 2009). The possible

explanation for such a high degree of parasitism in older animals may lie in a loss of immune response against previously exposed parasites (Traversa, 2012).

The observed low prevalence of tapeworm infections, specifically *D. caninum* and taeniid eggs, is likely underestimated due to the irregular release of tapeworm eggs from proglottids into the faecal mass, leading to false-negative results (Robertson et al., 2000). Furthermore, with only one case of each tapeworm detected in this survey, the limited number of positive samples is insufficient to conclude the influence of animal- and shelter-related variables on the presence of these GIP.

A single case of *E. granulosus* (s.l.) infection was detected in a geriatric dog from the Alentejo region that has, for a long time, the highest incidence of cystic echinococcosis in animals and humans in Portugal (Canhão-Dias et al., 2022, Alho et al. (2023). Alho et al. (2023) reported that each infected animal correlates with a 7 % increase in human hospitalization rates, underscoring the zoonotic link between animal and human health. Our finding emphasizes the need for regional epidemiological surveillance and targeted public health strategies, including effective parasite control of *E. granulosus* in dogs through regular administration of praziquantel, the drug of choice for cestode infections (ESCAPP, 2021), to mitigate the impact of this zoonotic disease.

5. Strengths, limitations, and recommendations for future studies

One of the major strengths of this study is its nationwide scope, providing valuable epidemiological data from official shelters serving 60 municipalities, thus representing a significant part of the country's shelter system and contributing to the understanding of GIP presence in shelter dogs across Portugal. Furthermore, focusing on dogs in official shelters provided insights into a particularly vulnerable population that often lacks prior veterinary care. Additionally, the use of Mini-FLOTAC, a validated and sensitive method for detecting GIP, ensured consistent diagnostic results across all samples.

Despite the above, certain regions are underrepresented, particularly areas south of the Tagus River, such as the Lisbon Metropolitan Area and the Algarve, as well as the Autonomous Region of Azores, which is only represented by the island of São Miguel. Including more shelters from these underrepresented areas in future studies would provide an even more comprehensive national perspective on GIP prevalence.

Furthermore, our conclusions regarding variables such as LOS and outdoor activity access are derived from a relatively small sample size that may have limited our ability to detect statistically significant associations.

The reliance on a single diagnostic method (Mini-FLOTAC), while effective, also presents certain limitations, particularly in its sensitivity for detecting protozoan infections like *G. duodenalis* and *C. canis*. Future studies should employ a combination of diagnostic techniques, including serological and molecular data, to provide a more comprehensive understanding of the prevalence of such parasites. Additionally, collecting samples over three consecutive days and analysing them by Mini-FLOTAC would increase the possibility of identifying positive cases for certain parasitic elements, particularly cysts of *G. duodenalis*, which are shed intermittently. Furthermore, these approaches would enhance the detection of low-intensity infections, and improve the overall accuracy of results.

Longitudinal studies could also provide a better understanding of how variables such as outdoor activity, deworming protocols, and shelter management practices influence parasite prevalence over time. Collecting more detailed metadata, such as the reproductive status of the dogs at admission (neutered/spayed or intact), timing of previous deworming treatments, date of shelter admission, geographical origin, and access to the outdoors during their stay, would further enhance our understanding of the factors that affect GIP prevalence.

Additionally, checking the registration status of animals in the SIAC

(“Sistema de Informação de Animais de Companhia”, Portugal’s national pet identification and registration system) could offer valuable insights into whether the animal might have received prior veterinary care. However, it is worth noting that while SIAC registration, micro-chipping, and rabies vaccination are mandatory, routine deworming is not typically recorded, which limits the reliability of SIAC as an indicator of previous deworming practices. Lastly, incorporating routine copro-parasitological analyses as part of the shelter management protocol could serve not only as a means of improving health management but also as a valuable data source for ongoing epidemiological surveillance.

Addressing these limitations and incorporating the recommended strategies will be essential for enhancing our understanding of GIP dynamics and improving the health outcomes of shelter dog populations.

6. Conclusions

This nationwide survey on parasitism in shelter dogs in Portugal provides valuable insights into the occurrence and regional variations of GIP, shedding light on the complex epidemiology of these parasitic infections and providing timely information for GIP prevention and control to veterinarians managing official shelters and other stakeholders.

The overall prevalence of GIP was lower than in previous studies, which may be partly attributed to methodological differences. Nevertheless, our findings, supported by similar results from a recent study (Rocha, 2022), suggest the effectiveness of internal deworming protocols combined with limited outdoor access. However, it is also essential not to disregard the significant role of proper hygiene and animal density management, as indicated by the results of this study. For instance, shelters with weekly walks showed lower GIP prevalence, highlighting that well-managed outdoor activities and stringent hygiene practices can effectively mitigate infection risks.

Despite these positive trends, overcrowded conditions in some shelters continue to pose a significant risk for the persistence and transmission of GIP, some of which are zoonotic. Coupled with inadequate sanitization, these conditions can facilitate the persistence and transmission of GIP. Thus, emphasizing proper sanitation measures, especially removing and disposing of faeces after deworming, is crucial in mitigating environmental contamination and infection risks for animals and staff.

In summary, this study provides essential data that can inform public health and shelter management policies, ultimately contributing to the control of GIP in vulnerable dog populations and reducing zoonotic risks.

CRedit authorship contribution statement

Patrícia Lopes: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jacinto Gomes:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **João Lozano:** Writing – review & editing. **Mariana Louro:** Writing – review & editing. **Luís Madeira de Carvalho:** Writing – review & editing. **Isabel Pereira da Fonseca:** Writing – review & editing, Resources. **Ricardo Lobo:** Writing – review & editing. **Fernando Monteiro:** Writing – review & editing. **Liliana Carvalho:** Writing – review & editing. **Paulo Afonso:** Writing – review & editing. **Miguel Almas:** Writing – review & editing. **Mónica V. Cunha:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization.

Ethical statement

The work described in the manuscript “Prevalence, diversity and risk factors of gastrointestinal parasites in dogs housed at official shelters across Portugal” involved using non-experimental animals only (shelter dogs) and non-invasive approaches were performed. Faecal sample

collection only started after obtaining informed consent from the local authorities. Veterinarians responsible for the official shelters randomly collected fresh faecal samples from the shelter grounds without interacting with or handling animals in a way that could cause them stress or harm. No invasive or painful procedures were performed on the dogs included in this study.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vprsr.2025.101285>.

Data availability

Data needed to evaluate the findings of this work are present in the paper and/or the Supplementary Materials. Any additional information is available from the corresponding author upon reasonable request.

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