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Original Article

Coprological survey of endoparasite infections in owned dogs and owners' perceptions of endoparasite control in Belgium and the Netherlands

L. Lempereur[a,*], R. Nijsse[c], B. Losson[b], F. Marechal[b], A. De Volder[b], A. Schoormans[c], L. Martinelle[d], F. Danlois[e], E. Claerebout[f]

[a] *FPS Health, Food chain Safety & Environment, Corporate Strategy, Place Victor Horta, 40 box 10, 1060 Brussels, Belgium*
[b] *Liège University, Faculty of Veterinary Medicine, Centre for Fundamental and Applied Research for Animal and Health (FARAH), Laboratory of Parasitology and Parasitic Diseases, 4000 Liège, Belgium*
[c] *Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, P.O. box 80.165, 3508 TD Utrecht, the Netherlands*
[d] *Liège University, Faculty of Veterinary Medicine, Experimental Station CARE – FePex, Center for Fundamental and Applied Research for Animal and Health (FARAH), 4000 Liège, Belgium*
[e] *Boehringer Ingelheim, Animal Health Belgium SA, Brussels, Belgium*
[f] *Ghent University, Faculty of Veterinary Medicine, Laboratory of Parasitology, Salisburylaan 133, B-9820 Merelbeke, Belgium*

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ABSTRACT

Infections with endoparasites are common in dogs. Some of these parasites are potentially zoonotic and therefore a public health concern. A survey was conducted in twenty-six small animal practices to evaluate the prevalence of endoparasites in Belgian and Dutch owned dogs older than 6 months as well as risk factors associated with infection. Out of 239 faecal samples screened (168 in Belgium and 71 in the Netherlands), 18 dogs were tested positive for at least one type of endoparasite with three dogs co-infected with two parasitic species. *Toxocara* sp. was the most frequently found endoparasite (4.6%). Three other dogs were positive for *Angiostrongylus vasorum* (1.4%) using the Baermann method and confirmed in one dog by the Angiodetect® test. Age and predation behaviour were identified as two risk factors associated with endoparasite infection. Although the majority (77%) of the owners in this study reported to administer at least one anthelmintic treatment per year, only a minority of them (24.3%) were aware of the risk to human health, indicating that owner awareness is sub-optimal. For dog owners, human toxocarosis and other potential zoonoses remain an underestimated health concern. The implementation of sustainable parasite control strategies should be promoted taking also into account the public health risk.

1. Introduction

Endoparasites are commonly found in dogs and are a point of concern for owners at veterinary appointments. Alongside the clinical signs that they can induce in dogs, some of these parasites are zoonotic and therefore of public health concern. *Toxocara canis* is one of the most common gastrointestinal parasites in Europe (Barutzki and Schaper, 2011; Claerebout et al., 2009; Symeonidou et al., 2017). Although the clinical impact of *T. canis* in adult dogs is usually limited, young dogs may be heavily infected showing signs such as vomiting and a distended abdomen. Additionally, the potentially severe zoonotic impact cannot be denied. Humans become infected following the ingestion of embryonated eggs (usually from soil-contaminated hands or food) or larval-contaminated undercooked meat; larvae then penetrate the intestinal mucosa causing an infection called *larva migrans interna*.

This infection can potentially induce visceral, ocular or neurological dysfunctions associated with a high level of eosinophils (Ma et al., 2018). From the zoonotic point of view, one of the most severe zoonotic canine endoparasites is represented by *Echinococcus multilocularis* (family *Taeniidae*) which may cause highly debilitating or fatal infections (Cambier et al., 2018; Deplazes et al., 2015). However, *E. multilocularis* eggs cannot be differentiated from other taeniid eggs using microscopy. Additionally, other endoparasites such as *Ancylostoma caninum* and *Dipylidium caninum* also pose zoonotic risks, however symptoms in people are typically far less severe (Landmann and Prociv, 2003; Raether and Hänel, 2003).

The French heartworm, *Angiostrongylus vasorum,* has been known for several years to be endemic in the Netherlands (van Doorn et al., 2009) and was recently reported in Belgium (Jolly et al., 2015). This lungworm is of clinical importance in dogs, causing mainly coughing

* Corresponding author.
E-mail address: laetitia.lempereur@health.fgov.be (L. Lempereur).

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and respiratory distress. Atypical signs include bleeding and neurological manifestations, however, some dogs may never develop clinical signs associated with this infection. The diagnosis is sometimes challenging as infected animals may exhibit clinical signs mimicking those of other conditions (e.g. immune-mediated conditions, Dirofilariasis, certain neoplasias) (Di Cesare et al., 2015). A confirmatory diagnosis can be obtained in-clinic using either the Baermann method to detect 1st stage larvae (L1) in the feces or a commercially-available test to detect circulating *A. vasorum* antigens in the blood (Schnyder et al., 2014). Several aspects, such as environmental and behavioural factors of dogs, are associated with the transmission and/or patency of these above mentioned parasites. These aspects need to be studied for a better understanding of the epidemiology and to evaluate the potential impact on animal and public health. Age and crowded living conditions are well-studied factors showing a predisposition for young and/or kennelled dogs to be infected by endoparasites (Claerebout et al., 2009; Nijsse et al., 2015; Riggio et al., 2013). Other conditions, such as walking off leash and predation behaviour, are associated with parasite infections as well (Nijsse et al., 2015). Further investigation of these factors could provide evidence to support effective control strategies.

The guidelines of the European Scientific Counsel Companion Animal Parasites (ESCCAP) currently recommend that adult dogs having access to the outdoors should be treated with an anthelmintic at least four times a year. The treatment frequency up to 12 times a year should be considered in situations where there is a high risk of human exposure (ESCCAP, 2017); alternatively, their infection status can be regularly monitored by faecal examination. The number of anthelmintic treatments to be applied is often controversial, and the practice of routine application of anthelmintics is questioned by some (Nijsse et al., 2016). Additionally, owner's compliance to recommended treatment strategies for dogs older than six months is often disappointing (Nijsse et al., 2015; Overgaauw et al., 2009). Therefore, the assessment of the risk factors combined with the compliance and the perception of the owner about dog anthelmintic treatment could provide useful information for the implementation of sustainable control.

This study was designed to evaluate the occurrence of endoparasites in Belgian and Dutch owned dogs older than 6 months as well as to identify associated risk factors. Additionally, the relationship between the historically practiced treatment frequency and the occurrence of patent infections was examined together with the owners' perception of the importance of endoparasite control.

2. Material and methods

2.1. Collection of samples and data

The survey was conducted from February to June 2017. Twenty-six small animal veterinary practices were selected on voluntary basis across Belgium and The Netherlands (Fig. 1).

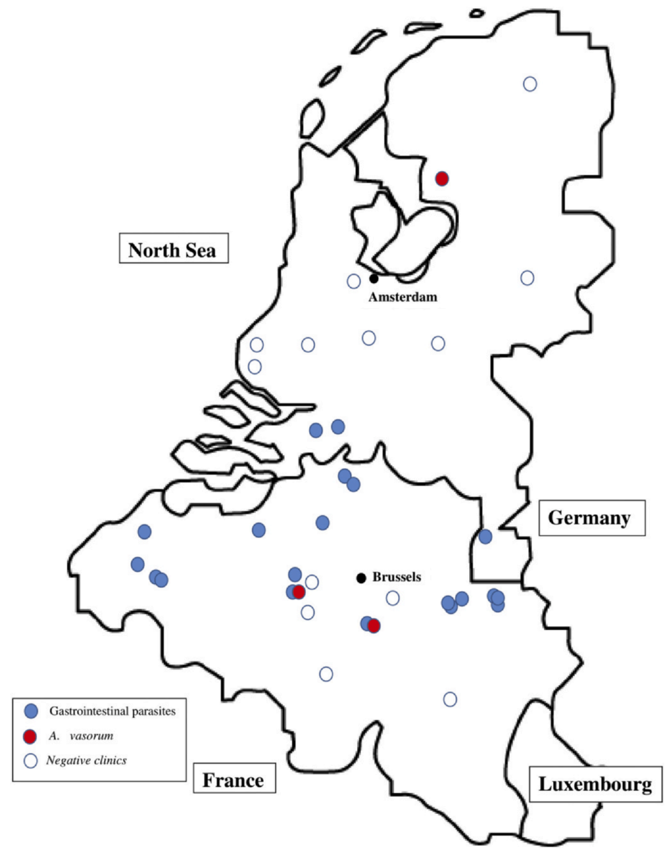

Fig. 1. Map of the 26 selected veterinary practices in Belgium and The Netherlands.

In each vet practice, blood samples were collected from dogs presented for various non-parasite related conditions (such as vaccination, sterilization, trauma, etc.), whereas faecal samples were brought by the owners. Dogs older than six months regardless of breed or sex were included in this survey. Exclusion criteria included use of anthelmintic treatment in the past two months or travel history outside Belgium and the Netherlands in the past three months. Simultaneously, a questionnaire was answered by the owner concerning sex, age, locality, life style of the dog, and recent anti-parasitic treatments.

2.2. Sample analyses

Recently voided canine faecal samples provided by the owners were evaluated for parasite stages by centrifugal flotation (4 *g* sample; $ZnSO_4$: s.g. = 1.3 g/cm3; 1000 RCF for 1 min) and by the Baermann larval migration-technique (2–20 g sample) when an adequate faecal volume was available. Parasite stages were identified according to Beugnet et al., 2018. Blood samples from these dogs were also collected in dry or heparinised tubes and centrifuged (2000 RCF for 10 min) in order to obtain serum or plasma, respectively. An in-clinic serological test detecting *A. vasorum* circulating antigen (Angiodetect®, IDEXX, Westbrook, ME, USA) was used for initial screening of all dogs following the manufacturer's instructions. This test has a reported sensitivity of 84.6% and a specificity of 100% (Schnyder et al., 2014). The reading of the test was performed strictly at 15 min according to the specifications provided by the manufacturer(Schnyder et al., 2014). All vet practice teams were trained and monitored in order to obtain comparable results.

2.3. Statistical analyses

To identify possible risk factors associated with parasite detection in dog feces, the answers to the questionnaire provided by each dog owner were encoded, and results of coprological tests were merged in an overall parasite infection status (positive or negative) as the binary outcome. The very few serological results were not statistically analysed. The host variables were made available to the model including sex, age, origin of the dog, episode of kennel housing, environment (urban, rural, forested or mixed), yearly frequency of anthelmintic treatment, time since the last anthelmintic treatment, potential contact with foxes, clinical signs indicative of a parasitic infection, predatory behaviour, average daily walking time, and owner's motivation to deworm. A univariate logistic regression analysis was conducted and odds ratio's (OR) with 95% confidence intervals (CIs 95%) were attributed to each variable. All variables with $p < 0.20$ in the univariate analysis were then included in a subsequent multivariate logistic regression analysis. The final model was chosen by AIC in a Stepwise Algorithm. A p value < 0.05 was used to define statistical significance.

Statistical analyses were performed using the R software/

Table 1
Parasites detected in canine faecal or *serum/plasma samples.

Parasite	n/N (%)	Belgium	The Netherlands
Toxocara sp.	11/239 (4.6)	10/168 (6)	1/71 (1.4)
Toxascaris leonina	1/239 (0.4)	0/168 (0)	1/71 (1.4)
Trichuris vulpis	3/239 (1.2)	3/168 (1.8)	0/71 (0)
Hookworms	2/239 (0.8)	1/168 (0.6)	1/71 (1.4)
Taeniidae	3/239 (1.2)	3/168 (1.8)	0/71 (0)
Dipylidium caninum	1/239 (0.4)	1/168 (0.6)	0/71 (0)
Toxocara/Taeniidae	2/239 (0.8)	2/168 (1.2)	0/71 (0)
Toxocara/*Trichuris*	1/239 (0.4)	1/168 (0.6)	0/71 (0)
A. vasorum Baermann	3/216 (1.4)	2/147 (1.4)	1/69 (1.4)
**A. vasorum* Angiodetect®	1/237 (0.4)	1/168 (0.6)	0/69 (0)

environment (R-3.5.1, R Foundation for Statistical Computing, http://www.r-project.org/).

3. Results

Out of the 239 faecal samples evaluated (168 from Belgium and 71 from the Netherlands), 18 (7.5%) dogs were found positive for at least one type of gastrointestinal parasite ova with 3 dogs (1.2%) co-infected with two parasitic species (Table 1). Eggs of *Toxocara* sp. (11/239, 4.6%) were the most frequently recovered parasite. Co-infections included the combination of *Toxocara* sp. with either Taeniidae eggs ($n = 2$) or *Trichuris* eggs ($n = 1$). Three dogs (3/216, 1.4%) with no evidence of gastrointestinal parasitism tested positive for L1 of *A. vasorum* using the Baermann technique; one of these dogs (1/237, 0.4%) was also positive by the Angiodetect® test (Table 1). None of the dogs with evidence of endoparasitism displayed clinical signs of an infection.

3.1. Risk factors

Presence of endoparasites was significantly different amongst age groups ($p = 0.009$ with 24% of dogs < 1 year (8/33) more frequently infected than those aged > 1 year (15/206, 7.3%) (Table 2). Owner perceived predatory behaviour was also significantly associated ($p = 0.018$) with a higher occurrence of endoparasites. Dogs participating in predation were more often infected (8/48, 16.6%) compared to dogs that did not participate in predation or dogs where the owner was unsure or unaware of predatory behaviour (15/191, 8%) (Table 2). No significant difference was found between female and male dogs ($p > 0.42$) nor in the infection rates of endoparasites in dogs living in rural, urban, forested or mixed environments ($p = 0.22$). Additionally, walking activity and the presence of foxes in the living environment were non-significant factors ($p = 0.08$) but were implemented in the multivariate analysis.

3.2. Anthelmintic treatment

Of the dogs with questionnaire data ($n = 220$) the owners of 35 dogs (15.9%) reported to never have administered anthelminthic drugs while 46 dogs (21%) were treated once a year, 70 (31.8%) twice a year, 39 (17.7%) three times a year, and 30 (13.6%) four times a year or more. Nineteen dogs (8%) had an unknown history of anthelminthic treatment. The anthelmintic treatment was not showing a trend ($p = 0.53$) of having an effect on the helminth occurrence no matter the reported treatment frequency (Table 2). In the univariate logistic regression analysis, ten variables were included (Table 2).

A stepwise approach was applied and demonstrated that the best predictive model utilized two variables, predation and presence of foxes in the environment ($p = 0.039$ and 0.22, respectively), with predation being the only significant factors.

Table 2
Risk factors for shedding parasite ova or *A. vasorum* L1.

Variable	Single-variable OR (95% CI; p)	Multivariable OR (95% CI; p)
Age class		
< 1 year old	Ref.	Ref.
> 1 year old	0.26 (0.09–0.074; 0.009)	0.24 (0.048–1.4; 0.09)
Sex		
Female	Ref.	
Sterilised female	0.34 (0.065–1.64; 0.18)	
Male	1.21 (0.35–0.26; 0.78)	
Neutered male	1.4 (0.45–0.58; 0.6)	
Location origin		
The Netherlands	Ref.	
Belgium -Ghent	1.58 (0.45–6.3; 0.48)	
Belgium- Liege	1.62 (0.5–6.22; 0.44)	
Treatment frequency per year	1.12 (0.77–1.63; 0.53)	
Time since last deworming	0.98 (0.90–1.03; 0.68)	
Dog displaying associated clinical signs	0.17 (0.009–0.87; 0.09)	0,49 (0.02–3.24; 0.53)
Presence of foxes in living environment	2.33 (0.7–10.61; 0.2)	1.45 (0.28–10.7; 0.67)
Preying on small animals	2.89 (0.98–8.25; 0.047)	1.4 (0.24–6.7; 0.68)
Average daily walking duration (min)	1.003 (1.003–1.01; 0.047)	1.001 (0.999–1.0037; 0.1)
Environment		
Forested	Ref.	
Urban	1.9 (0.3—3.6; 0.57)	
Urban mixed rural	2.8^{-6} (NA—1658; 0.99)	
Rural	1.2 (0.2—23; 0.87)	
Rural mixed forested	4,3 (0.5—8.9; 0.22)	

3.3. Owners' perceptions toward anthelmintic treatment

Majority of owners using anthelmintic drugs (147/206, 71.4%) reported the dog's health as the main reason for treating, while 1.5% (3/206) were mainly concerned about the public health issue (Table 3) and treated their dog once or twice a year. Forty seven owners (47/206, 22.8%) described both dog and human health as the main reasons. A relatively low number of owners (9/206, 4.4%) reported annual use of an anthelmintic. Many owners (45/185, 24.3%) were not able to communicate the name of the last anthelmintic drug administered to their dog while 58.4% (108/185) were using a drug combination of praziquantel with milbemycin oxime (87/185, 47%) or pyrantel (21/185, 11.3%).

4. Discussion

The infection rates of endoparasites in dogs reported in this study are in accordance with previous surveys looking at owned dogs from the same regions (Claerebout et al., 2009; Nijsse et al., 2015; Overgaauw et al., 2009) and from other European countries (Kostopoulou et al., 2017; Paoletti et al., 2015; Raue et al., 2017). The relatively low occurrence of detected parasites in this study is not surprising as this study focused on owned dogs which seem to have fewer infections compared to kenneled or unowned dogs (Claerebout et al., 2009). Actually, the number of dogs having spent a period of time in a kennel included in this survey was negligible (6/239, 2.5%).

Eggs of *Toxocara* sp. were recovered most frequently in this study. The prevalence of *Toxocara* sp. could be sometimes overestimated in the case of coprophagic dogs as it can be difficult to determine if eggs are present due to a patent infection or secondary to coprophagy or predation (Fahrion et al., 2011; Nijsse et al., 2014). The morphological differentiation based on the egg surface is not always successful using light microscopy (Uga et al., 2000); additionally the intestinal transit

Table 3
Owner reasons for anthelmintic treatment and applied treatment frequencies in individual dogs.

Treatment frequency per year	Routine use	Dog's health	Combination	Public health	Total n (%)
1	6	37	8	1	52 (25.2)
2	1	52	23	2	78 (37.9)
3	2	34	9	0	45 (21.8)
4	0	23	7	0	30 (14.6)
6	0	1	0	0	1 (0.5)
Total n (%)	9 (4.4)	147 (71.4)	47 (22.8)	3 (1.4)	206

may alter the eggs making differentiation more difficult. On the other hand as dogs under 6 months of age were excluded from the study this could also have led to and underestimation of *Toxocara* sp.

Trichuris vulpis was rarely observed in owned dogs and the percentage observed in this study is in accordance with other studies (Deplazes et al., 2016). It is noteworthy that a flotation solution with a high specific gravity (≥ 1.3) is ideal for the detection of this type of egg. Hookworms were observed in 0.8% of the samples. In Belgium and the Netherlands *Ancylostoma caninum* is not recorded as indigenous and consequently these dogs were infected most likely with *Uncinaria stenocephala*. *Dipylidium caninum* was rarely observed (0.4%) and this is due to the active shedding of mature proglottids most of the time apart from defecation. Taeniidae eggs were detected in 1.2% of the samples. The potential zoonotic impact of this observation is difficult to assess as *E. multilocularis* eggs must be identified though molecular techniques.

The diagnosis for *A. vasorum* was evaluated using both the Baermann method on a faecal sample and the Angiodetect® test on serum or plasma. In this study, of the three dogs shedding *A. vasorum* larvae in feces, only one had detectable antigen in the blood sample. The discrepancy between the results obtained from these two tests could be explained by the earlier detection via the Baermann method (6–7 weeks post infection [PI]) compared to the Angiodetect® test (9 weeks PI) (Schnyder et al., 2014). *Crenosoma vulpis*, another canine lungworm species, was not observed in this study, even though this parasite is known to be present in the region (Borgsteede, 1984; Lempereur et al., 2016). Therefore, *C. vulpis* infection should not be omitted as a potential differential diagnosis in dogs, and careful examination of any L1 in the feces should be made via the Baermann method; solely performing the Angiodetect® for a suspected lungworm infection would fail to detect *C. vulpis* as the test is *A. vasorum* specific (Lempereur et al., 2016; Schnyder et al., 2014).

The analysis of the questionnaires revealed two risk factors associated with endoparasite infection: age and predatory behaviour. Both criteria are often described as risk factors (Bajer et al., 2011; Claerebout et al., 2009; McNamara et al., 2018; Nijsse et al., 2015) although comparison between studies remains sometimes difficult due to unharmonized criteria. Younger age has unsurprisingly been reported as a risk factor for parasite infections, however the minimum age for dogs in this study was 6 months. This observation confirms that age resistance, a phenomenon described as a decrease in egg-shedding or the recovery rate of adult ascarids as the age of the dog advances (Greve, 1971; Overgaauw and Nederland, 1997) is a progressive process and consequently this factor was difficult to address fully in the present study.

However, patent *Toxocara canis* infections may still occur in adult dogs following ingestion of infective eggs (Dubey, 1978), ingestion of infective larvae in paratenic hosts (Strube et al., 2013), or immune response deficiency (Fahrion et al., 2008). Additionally, fully susceptible adult dogs have also been described, despite repeated egg exposure and development of antibodies (Maizels and Meghji, 1984). Besides age, predation was also demonstrated to be a risk factor in our study. With the ingestion of infective larvae in paratenic hosts as a potential route of infection (Strube et al., 2013), there is the potential for larvae to mature in the dog's intestine without completing the hepato-tracheal migration, therefore, evading the dog's immune system. Other influencing factors have been described in previous studies, such as outdoor access, especially off-leash time (McNamara et al., 2018; Nijsse et al., 2015), however this factor was not significant in our study. Although the majority (77%) of owners in this study reported to apply at least one anthelmintic treatment per year, this was mainly for the health of their dog. Only a minority of them (24.3%) were aware of any zoonotic risk. Indeed human risk exists for some parasites, especially *Toxocara* sp. Serological surveys in the Netherlands showed a relatively high seroprevalence of *Toxocara* antibodies in humans (Mughini-Gras et al., 2016) while data on human seroprevalence for *Toxocara* sp. in Belgium are not available. However, a recent study indicated that 14% of public sandpits were contaminated with *Toxocara* sp. eggs (Vanhee et al., 2015). This demonstrates that environmental contamination with *Toxocara* sp. exists in urban areas in Belgium, even if cats seem most likely to be the main source (Morgan et al., 2013; Vanhee et al., 2015). Other parasites with zoonotic potential, such as hookworms and some cestodes, were also investigated in this study, but showed a relatively low prevalence. It is noteworthy that even those owners who indicated that they treated their dog for public health purposes (1.5%) were not following the ESCCAP recommendations regarding treatment frequency. These data indicate that owner awareness and compliance are not optimal, and that despite the availability of effective anthelmintic treatments for pets, toxocarosis and other potential zoonoses remain a potential health concern for pets and people. ESCCAP guidelines advocate a dosing frequency of four times a year in dogs in order to reduce adults from shedding eggs resulting in environmental contamination. However, this recommendation may not prevent a patent infection as the prepatent period is approximately 1 month. Additionally, in this study the anthelmintic treatment was not associated with an effect on the helminth occurrence which was already described in a previous study (Sager et al., 2006). On the other hand, several studies indicate that a majority of adult dogs rarely have patent infections with *T. canis* (Claerebout et al., 2009; Nijsse et al., 2015; Overgaauw et al., 2009). Therefore, risk assessment and regular faecal examinations for individual dogs may provide a more customized parasite control program. In this study, only 11.8% (27/229) of the sampled dogs had previously had a coprological examination in the past, indicating that faecal examinations are not a common practice for companion animals.

5. Conclusions

The implementation of sustainable antiparasitic strategies should be promoted, taking into account both animal health and public health risk. Furthermore, veterinarians should play a key role in raising awareness in pet owners regarding prevention of endoparasite infections.

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Ethical statement

Blood samples from dogs were collected by veterinarians and followed the International Guiding Principles for Biomedical Research

Involving Animals to ensure animal welfare.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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