

## Original Article

## *Toxascaris leonina* in dogs – A nematode species of high prevalence in some regions of Eurasia

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## ABSTRACT

A recent meta-analysis of studies from around the world had shown a global prevalence of *Toxascaris leonina* in stray dogs of about 7%. However, studies from Eurasian regions, where higher percentages of positive dogs were often found, were under-represented in this meta-analysis. This prompted the present study. Its main objective was to examine free-roaming dogs from the capital city of Kazakhstan to obtain information on the current occurrence of *T. leonina* egg shedding compared to *Toxocara* spp.

Faecal samples from 500 free-roaming dogs from the city of Astana and its suburbs were collected 1–2 days after admission to the municipal animal shelter during three time periods (May to October 2019, September 2020, and April 2021). Samples were examined by a saline flotation method. Nematode egg-positive samples were subjected to a modified McMaster method to determine the number of eggs per gram of faeces (EPG).

*Toxascaris leonina* eggs were found in 53.8% and *Toxocara* spp. eggs in 14.8% of the dogs examined. The prevalence of *T. leonina* egg shedding was significantly associated with age class but not with sex: dogs aged between 6 months and 2 years were more likely to be *T. leonina* egg positive than puppies. The intensity of *T. leonina* egg shedding was also age dependent: dogs aged between 6 months and 2 years had significantly higher EPGs compared to younger or older animals. In contrast, the prevalence of *Toxocara* spp. egg shedding was associated with both age class and sex: dogs older than 2 years were less likely to shed *Toxocara* spp. eggs than puppies, and females were less likely to be *Toxocara* spp. egg positive compared to males.

The present results confirm those of other studies in Eurasian regions. It is therefore reasonable to assume that the global prevalence of *T. leonina* infection has been underestimated and should be revised accordingly.

### 1. Introduction

*Toxascaris (T.) leonina* LEIPER 1907 is one of the ascarid species that parasitise the intestines of canids and felids as definitive hosts (Deplazes et al., 2016). Unlike *Toxocara* species, *T. leonina* is considered a negligible parasite from a veterinary and zoonotic point of view. Unambiguous cases of clinical toxascariosis are neither known in dogs (Georgi and Georgi, 1992) nor in cats (Rostami et al., 2020b). However, *T. leonina* has been reported to affect body weight and fur quality in heavily infected arctic foxes (*Vulpes lagopus*) under experimental conditions (Anikieva et al., 2012). This parasite has little or no zoonotic

relevance (Rausch and Fay, 2011) but occurs worldwide. Therefore, its detection can be used as an indicator of environmental contamination with exogenous parasite stages. In a meta-analysis of records from 40 countries its ‘pooled’ global prevalence was estimated at 2.9% in dogs, including 7.0% in strays (Rostami et al., 2020b). It is striking that the Eurasian regions were under-represented in this meta-analysis. An analysis of reports from these regions showed that the percentage of free-roaming dogs testing positive for *T. leonina* was often much higher. This is illustrated by the following examples: up to 38% in eastern Türkiye (Balkaya and Avcioglu, 2011), 53% in western Iran (Adinezhadeh et al., 2013), 57% in Caucasian Russia (Trunova, 2008), 14% in

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Central Russia (Balandina et al., 2014), 23–40% in western, central and northern regions of Kazakhstan (Lider et al., 2010; Valieva, 2012; Sul-tanov et al., 2014), 89% in Uzbekistan (Safarov et al., 2022), and 32% in the Hunan province of China (Dai et al., 2009).

The capital city of Kazakhstan, Astana, is known for its large population of free-roaming dogs and cats (animals being not under direct human control): in 2010, over 3100 free-roaming dogs were caught and >2300 dog carcasses collected (Kusainova et al., 2011), and, in 2020, about 5200 free-roaming dogs and cats were captured and handed over to the municipal animal shelter, according to official data (Anonymous, 2021). The main objective of the present study was to obtain information on the current occurrence of *T. leonina* egg shedding compared to *Toxocara* spp. in free-roaming dogs from the capital city of Kazakhstan.

## 2. Materials and methods

### 2.1. Study location, animals, and sampling

This cross-sectional study was conducted in Astana in north-central Kazakhstan, which has a humid continental climate with warm summers and long cold winters, with no major differences in precipitation between the seasons (“Dfb” climate zone according to the Köppen-Geiger classification; Beck et al., 2018).

A total of 500 free-roaming dogs were sampled over three time periods: between May and October 2019 ( $N = 393$  dogs), in September 2020 ( $N = 47$ ) and in April 2021 ( $N = 60$ ). The dogs, almost all mongrels, had been captured in the city and its suburbs by the ‘Astana Vet-service’, brought to the municipal animal shelter and housed in individual cages. Fresh faecal samples were collected 1–2 days after admission to the shelter and prior to deworming. Dogs were categorized by sex and estimated age class (3 to 6 months; > 6 months to 2 years; > 2 years) based on dentition (Gracis, 2018).

### 2.2. Laboratory methods

Within one day of collection, faecal samples were examined macroscopically for cestode proglottids followed by the qualitative direct flotation method using saturated sodium chloride solution (specific gravity: 1.2) as flotation fluid for worm eggs and coccidian oocysts (Deplazes et al., 2016). Identification of parasite stages was based on morphological characteristics (Deplazes et al., 2016). Nematode egg-positive samples were then subjected to a modified McMaster method to determine the number of eggs per gram of faeces (EPG), which allows a detection limit of 50 EPG (Deplazes et al., 2016). Samples with no nematode eggs counted by the McMaster method but positive by the qualitative direct flotation method were assigned an EPG of 25 (half the detection limit of the McMaster method) for the calculation and presentation of results.

### 2.3. Statistical analyses

An explorative data analysis was performed using the R software environment for statistical computing and graphics (R-Core-Team, 2021); Rstudio served as editor, and the R libraries “epiDisplay”, “lme4”, “rms”, “jsmodule” and “aod” were applied. The prevalence was calculated as described by Bush et al. (1997). The influence of sex and age class on *T. leonina* and *Toxocara* spp. egg shedding was statistically analysed using bivariable analysis performed by logistic regression as described elsewhere (Maksimov et al., 2017). Briefly, the variables sex and age class were tested against positivity status using the R package “stats”. In addition, a multivariable analysis was performed, where variables with  $P$  values  $\leq 0.2$  in the bivariable analysis were included in the full model. Associations between egg counts (*T. leonina*, *Toxocara* spp.) and sex or age class were tested for significance using the Median test or Kruskal–Wallis test followed by the Dunn test for pairwise comparison of groups, respectively. Positive ascarid egg counts in each age

class were grouped into three EPG categories (>0 to 150, 200–450 and  $\geq 500$  EPG). Groups were compared using the Chi-square test. Any effects of the variables sex and age on cestode or *Cystoisospora* infection were tested for significance using the Chi-square test. Differences with  $P$  values  $< 0.05$  were considered statistically significant.

## 3. Results

*Toxascaris leonina* eggs were found in 53.8% and *Toxocara* spp. eggs in 14.8% of the 500 free-roaming dogs examined. Simultaneous shedding of *T. leonina* and *Toxocara* spp. eggs was observed in 8.2% of dogs. Other parasite stages detected were taeniid eggs and/or *Taenia* sp. proglottids, *Dipylidium* sp. egg capsules and/or proglottids, *Cystoisospora canis* oocysts and, in one dog each, hookworm and whipworm eggs (Table 1).

The prevalence of *T. leonina* egg shedding was significantly associated with the age class but not with the sex (Table 2): dogs aged between 6 months and 2 years were more than twice as likely (odds ratio 2.34) to be *T. leonina* egg positive than puppies. Unfortunately, it was not possible to perform the multivariable logistic regression on the *T. leonina* data, because the sex variable, which had a  $P$  value  $> 0.2$  in the bivariable analysis (Table 2), had to be excluded from the model. The intensity of *T. leonina* egg shedding was also age dependent (global  $P$  value  $< 0.001$ ): dogs aged >6 months to 2 years had significantly higher EPGs on average (arithmetic mean  $\pm$  standard deviation:  $439 \pm 634$ ) than younger or older animals ( $173 \pm 364$  and  $296 \pm 540$ , respectively; Fig. 1A). The *T. leonina* egg count was  $\geq 200$  EPG in 46% of all positive puppies; 35% of the puppies had  $\geq 500$  EPG. In older dogs, egg counts of  $\geq 200$  EPG and  $\geq 500$  EPG were found in 62–65% and 49–56% of positive cases, respectively (Fig. 2A). There was no significant difference in the EPG categories of positive dogs between the three age classes (Fig. 2A).

In contrast, the prevalence of *Toxocara* spp. egg shedding was significantly associated with both age class and sex; the multivariable logistic regression model showed that this was true for both variables independently of each other (Table 3): dogs >2 years of age were approximately two-thirds less likely (odds ratio 0.61) to shed *Toxocara* spp. eggs than puppies, and females were on average approximately half as likely (odds ratio 0.55) to be *Toxocara* spp. egg positive than males. The intensity of *Toxocara* spp. egg shedding tended to decrease on average, but not significantly, with increasing age class (Fig. 1B). The *Toxocara* spp. egg count was  $\geq 200$  EPG and  $\geq 500$  EPG in 65% and 35% of the positive puppies, respectively. In older dogs,  $\geq 200$  EPG was found in 57–60% of positive cases and  $\geq 500$  EPG in 27–30% (Fig. 2B). The EPG categories of positive dogs did not differ significantly between the three age classes (Fig. 2B).

The excretion of faecal stages of taeniids, *Dipylidium* sp., or *C. canis* was not significantly associated with sex or age class.

## 4. Discussion

The main objective of this study was to obtain information on the

**Table 1**

Prevalence of parasite stages in faecal samples from 500 free-roaming dogs examined 1–2 days after admission to the municipal animal shelter in Astana, Kazakhstan.

Parasite stage	No. positive	% positive (95% CI)
<i>Toxascaris leonina</i> eggs	269	53.8 (49.3–58.2)
<i>Toxocara</i> spp. eggs	74	14.8 (11.8–18.2)
Hookworm eggs	1	0.2 (0–1.1)
<i>Trichuris</i> sp. eggs	1	0.2 (0–1.1)
Taeniid eggs/ <i>Taenia</i> sp. proglottids	24	4.8 (3.1–7.1)
<i>Dipylidium</i> sp. egg capsules/proglottids	10	2.0 (1.0–3.6)
<i>Cystoisospora canis</i> oocysts	13	2.6 (1.4–4.4)

95% CI: 95% confidence interval.

**Table 2**

Effect of sex and age class on the prevalence of faecal *Toxascaris leonina* egg shedding in free-roaming dogs examined 1–2 days after admission to the municipal animal shelter in Astana, Kazakhstan (bivariable analysis by logistic regression).

Variable	No. examined	No. positive	% positive	Odds ratio (95% CI)	P value (LR test)	P value (Wald test)
Sex					0.395	
Male	108	62	57.4	Ref.		
Female	392	207	52.8	0.83 (0.54–1.28)		
Age class					< 0.001	
3 to 6 m	160	69	43.1	Ref.		
>6 m to 2 y	211	135	64.0	2.34 (1.54–3.57)		< 0.001
>2 y	129	65	50.4	1.34 (0.84–2.13)		0.219

95% CI: 95% confidence interval; LR test: likelihood-ratio test; m: months; y: years; Ref.: reference group.

occurrence of *T. leonina* infection in comparison to *Toxocara* spp. in free-roaming dogs using coprological methods. In this context, it should be noted that the detection of ascarid eggs (and other parasite stages and species) in the dog faeces may be a false positive result due to coprophagy. For example, dogs often shed eggs of the feline roundworm, *Toxocara cati*, as a result of intestinal passage of ingested eggs (Fahrion et al., 2011; Nijse et al., 2014; Vienazindienė et al., 2018). Coprophagy is a common behaviour in domestic dogs of all ages (Hart et al., 2018). Although the frequency of coprophagy in (adult) stray dogs is not known, it is likely to be as common as in pet dogs. False positive results could be excluded, for example, by repeating faecal examinations at short intervals: in the case of coprophagically ingested worm eggs, repeated faecal samples would usually be negative (Nijse et al., 2014). Ingestion of eggs from the environment by coprophagy would be expected to result in a rather low egg count, although it is not impossible that an individual dog could ingest canine or feline faeces with a high concentration of eggs, resulting in the excretion of many passage eggs. In the present study, dogs were examined only once, but quantitatively by determining the number of eggs in faeces. Low egg counts (<200 EPG) of both ascarids were present in approximately 40% of all egg-positive dogs. However, a low level of egg shedding is not necessarily an indication of a false positive result. Therefore, although false positives are likely to have occurred, they are not considered to be an important bias in the present study. This also means that the *Toxocara* spp. eggs found in the majority of dogs are considered to be those of *Toxocara canis*, although it cannot be excluded that in some cases *Toxocara* spp. eggs found in the sample were ingested and passaged *Toxocara cati* eggs. In future studies, it would be advisable to identify *Toxocara cati* eggs by measuring the egg size (*Toxocara cati* eggs are slightly, but significantly, smaller than *Toxocara canis* eggs) or using molecular biology methods (Fahrion et al., 2011; Vienazindienė et al., 2018).

#### 4.1. Prevalence of *T. leonina* infection

*Toxascaris leonina* was the dominant intestinal nematode species in free-roaming dogs in Astana. Its prevalence was substantially higher than that reported for free-roaming dogs in many other countries (4.3–10.3%; Rostami et al., 2020b). Necropsy studies had already shown a comparatively high prevalence of this roundworm species in free-roaming dogs from northern, central and western Kazakhstan (23–40%; Lider et al., 2010; Valieva, 2012; Sultanov et al., 2014) and in wolves from southern Kazakhstan (37%; Abdybekova and Torgerson, 2012). Many *T. leonina*-positive free-roaming dogs had been also

reported from neighbouring regions such as western Siberia (40%; Ponamarev and Luneva, 2013) and Uzbekistan (89%; Safarov et al., 2022). Therefore, the present results and those of other studies in Eurasian regions make it likely that the global prevalence of *T. leonina* estimated in the meta-analysis by Rostami et al. (2020b) is biased and obviously underestimated; the conclusions on risk factors drawn there should therefore be revised accordingly.

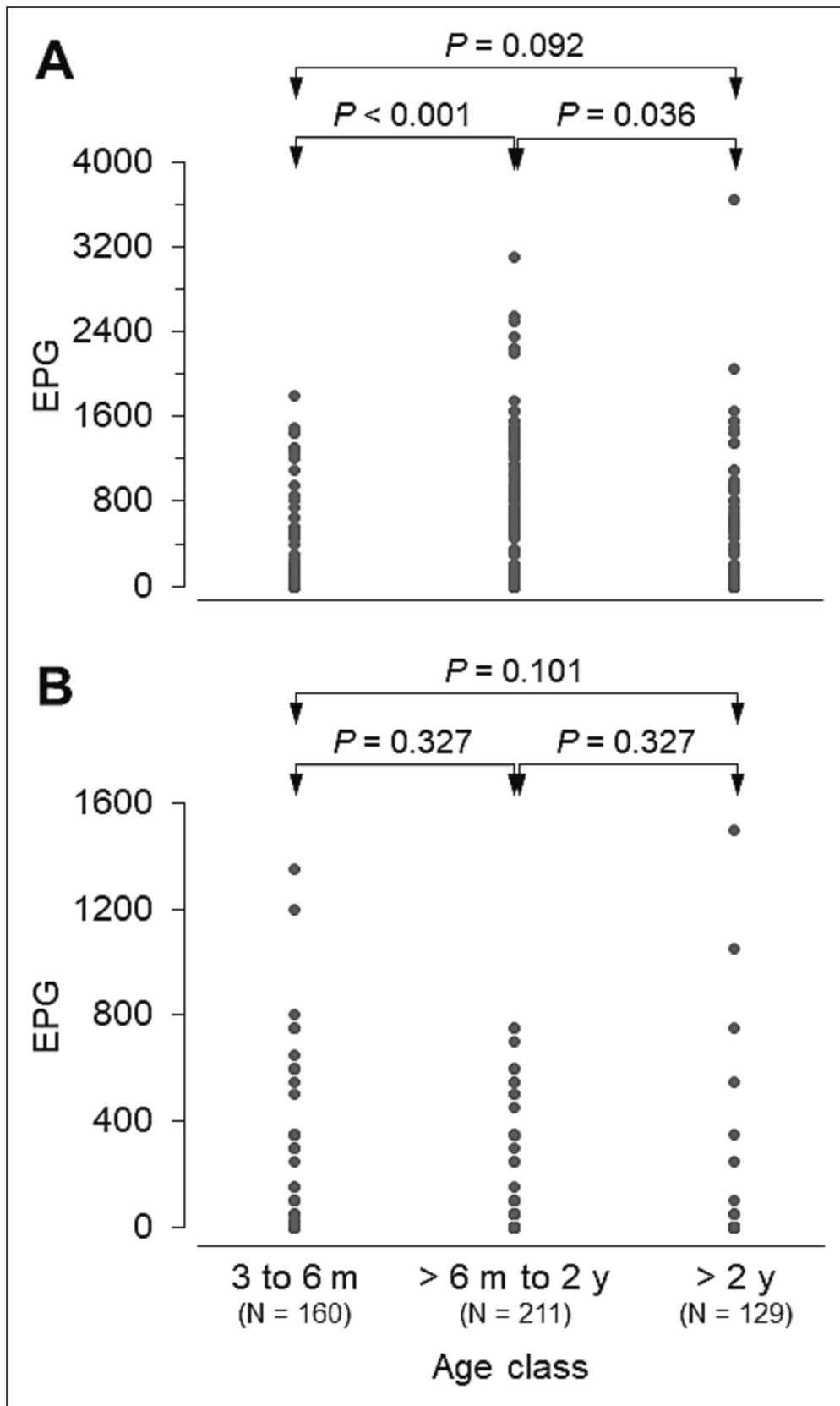
#### 4.2. Climatic conditions and ascarid infection

It is noteworthy that high prevalences of *T. leonina* infection have been recorded in free-roaming dogs from very different climatic zones of Eurasia (Köppen-Geiger climate zone classification; Beck et al., 2018): cold desert (“BWk”; Safarov et al., 2022) and cold semi-arid zones (“BSk”; Trunova, 2008), humid continental zones with long, cold winters and warm summers (“Dfb”; Lider et al., 2010; Balkaya and Avcioglu, 2011; Ponamarev and Luneva, 2013; Balandina et al., 2014; present study) or hot summers (“Dfa”; Valieva, 2012), and hot Mediterranean summer (“Csa”; Safarov et al., 2022) and humid subtropical zones (“Cfa”; Dai et al., 2009). However, the data show that *T. leonina* is particularly common in regions with harsh and/or cold climates. This is supported by studies in subarctic, paleartic and nearctic regions (“Dfc” or “ET” climate zone). There, *T. leonina* was found to be a dominant or even the only roundworm species in free-roaming dogs (Unruh et al., 1973; Rausch and Fay, 2011) and wild canids such as arctic fox and wolf (*Canis lupus*) (e.g., Skirnisson et al., 1993; Kapel and Nansen, 1996; Craig and Craig, 2005; Elmore et al., 2013; McGrew et al., 2015; Andreassen et al., 2017; Bouchard et al., 2021). Although *T. leonina* isolates from wolves and dogs, on the one hand, and from foxes, on the other hand, belong to phylogenetically different species clades (Fogt-Wyrwas et al., 2019; Xie et al., 2020), they do not seem to differ from each other in this regard.

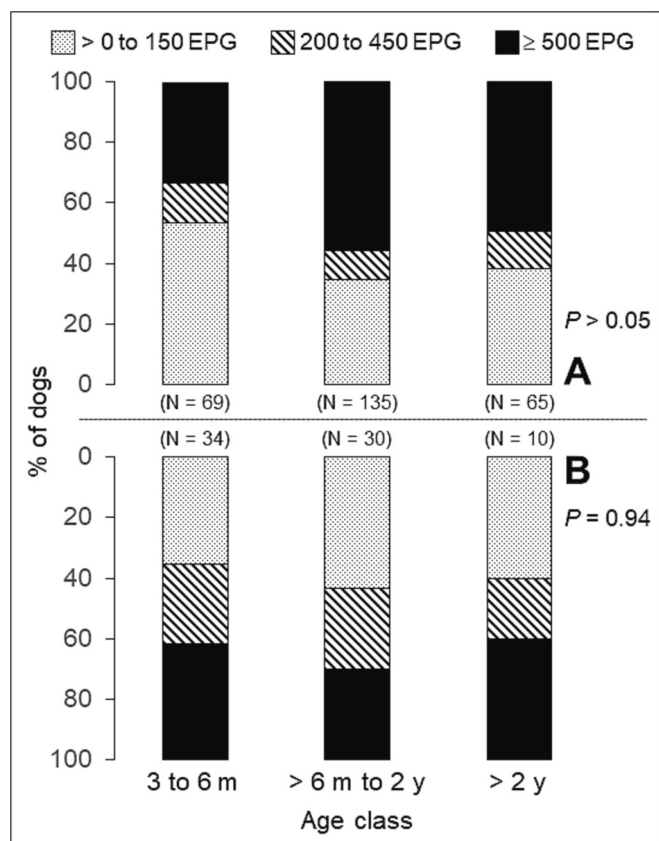
There are two plausible explanations why *T. leonina* is particularly common in regions with harsh, cold climates compared to *Toxocara canis*. First, it may be because *T. leonina* eggs are more cold-tolerant than *Toxocara* spp. eggs. After prolonged storage at freezing temperatures, *T. leonina* eggs develop normally at room temperature and embryonated eggs remain alive, whereas most *Toxocara canis* eggs die within a few days (Okoshi and Usui, 1968a; O’Lorcain, 1995). According to Kazanina (2014), *T. leonina* eggs excreted in winter do not develop but continue to be viable and develop into the infective stage in the following spring. Another explanation could be the different routes of infection of the two ascarid species: in *Toxocara canis*, the vertical (i.e., transplacental) transmission of third-stage larvae from the infected bitch to the fetus is considered to be the epidemiologically most important route because very efficient (Schnieder et al., 2011). In contrast, *T. leonina* does not have a vertical but only a horizontal route of infection by ingestion of embryonated eggs from the environment or third-stage larvae in the tissue of infected rodent prey (paratenic host) (Matoff and Wassileff, 1958; Sprent, 1959; Okoshi and Usui, 1968b). The latter is presumably the main route of infection in free-roaming dogs and wild canids in cold regions (Craig and Craig, 2005; Rausch and Fay, 2011; Bouchard et al., 2021) and therefore favorizing egg excretion with *T. leonina* eggs.

#### 4.3. Host age and ascarid infection

In this study, *T. leonina* eggs were found in dogs as young as 3–6 months old, but both its prevalence and intensity were higher in animals aged 6 months to 2 years. Its intensity was also higher in the middle age class than in older animals. These results may contrast somewhat with those from necropsies of arctic foxes, where the number of *T. leonina* worms was significantly higher in juveniles under one year of age than in older animals (Kapel and Nansen, 1996). Nevertheless, the present findings could be explained as follows: dogs, as altricial animals, are only sporadically in contact with *T. leonina* eggs from the environment in the first few weeks after birth (Fisher et al., 2002), with exposure



**Fig. 1.** Egg counts (EPG) of *Toxascaris leonina* (A) and *Toxocara* spp. (B) in 500 free-roaming dogs, depending on age class, examined 1–2 days after admission to the municipal animal shelter in Astana, Kazakhstan (m: months; y: years; N: number of dogs examined; P values by Kruskal-Wallis test followed by Dunn test).



**Fig. 2.** Percentage (%) of egg count (EPG) categories in *Toxascaris leonina* (A) and *Toxocara* spp. (B) egg-positive free-roaming dogs, depending on age class, examined 1–2 days after admission to the municipal animal shelter in Astana, Kazakhstan (m: months; y: years; N: number of positive dogs; P values by Chi-square test).

increasing after weaning (Jacobs et al., 1988), leading to start of egg shedding after a prepatent period of 7–11 weeks (Matoff and Wassileff, 1958; Sprent, 1959; Okoshi and Usui, 1968b). In addition, it was observed that experimentally infected puppies under 2 months of age were less susceptible to *T. leonina* infection than dogs over 1 year of age (Matoff and Wassileff, 1958), which may indicate a kind of juvenile resistance to this nematode. The frequency and intensity of infection may increase with age when rodents are consumed. Their contribution as paratenic hosts to the population dynamics of this ascarid species has already been hypothesised in the red fox (*Vulpes vulpes*) (Reperant et al., 2007). Finally, the tendency for lower *T. leonina* egg shedding in dogs >2 years of age may be interpreted as a result of acquired partial immunity to this roundworm species, as has been seen after reinfection under experimental conditions (Okoshi and Usui, 1968b).

In contrast to *T. leonina*, *Toxocara* spp. eggs, which we consider to be

**Table 3**

Effect of sex and age class on the prevalence of faecal *Toxocara* spp. egg shedding in free-roaming dogs examined 1–2 days after admission to the municipal animal shelter in Astana, Kazakhstan.

Variable	No. examined	No. positive	% positive	Odds ratio (95% CI)	Bivariable analysis by logistic regression		Multivariable logistic regression model	
					P value (LR test)	P value (Wald test)	P value (LR test)	P value (Wald test)
Sex					0.038		0.022	
Male	108	23	21.3	Ref.				
Female	392	51	13.0	0.55 (0.32–0.95)				
Age class					0.005		0.003	
3 to 6 m	160	34	21.3	Ref.				
>6 m to 2 y	211	30	14.2	0.61 (0.36–1.06)		0.077		0.058
>2 y	129	10	7.8	0.31 (0.15–0.66)		0.002		0.001

95% CI: 95% confidence interval; LR test: likelihood-ratio test; m: months; y: years; Ref.: reference group.

mainly those of *Toxocara canis*, were shed significantly more frequently in puppies than in older dogs. This is a common, well-known finding (Rostami et al., 2020a). It can be explained by the epidemiologically important transplacental route of *Toxocara canis* infection, which results in egg shedding starting a few weeks post partum, and the later development of partial immunity in adult canids (Schneider et al., 2011).

4.4. Host sex and ascarid infection

The prevalence of *T. leonina* egg shedding did not differ between the sexes in this study. The few relevant studies in the literature also show no difference between males and females in free-roaming dogs (Balkaya and Avcioglu, 2011; Adinezadeh et al., 2013) or red foxes (Tylkowska et al., 2021). In contrast, for *Toxocara* spp., male dogs were more likely to be egg positive than females, regardless of age class. This is consistent with the results of many other studies (Rostami et al., 2020a). The biological relevance of this finding is still unclear (Schneider et al., 2011) and remains to be determined.

4.5. Prevalence of other parasitic infections

The saline flotation method used to detect ascarid eggs is also suitable for the detection of faecal stages of hookworms and coccidia (Deplazes et al., 2016). The rare detection of hookworm eggs (presumably those of *Uncinaria stenocephala*) is consistent with results from previous necropsy studies in Kazakhstan (0–2%; Lider et al., 2010; Valieva, 2012; Sultanov et al., 2014). In other Eurasian regions, *U. stenocephala* was reported to be more prevalent, e.g. in Uzbekistan (37%; Safarov et al., 2022), Caucasian Russia (23%; Trunova, 2008) and Central Russia (52%; Balandina et al., 2014). The reasons for these regional differences remain to be investigated.

Data on the occurrence of canine *Cystoisospora* spp. infections from Eurasian regions, especially Central Asia, are scarce (Dubey and Lindsay, 2020). In the present study, *C. canis* oocysts were detected in a few dogs. This certainly does not reflect its true prevalence as the majority of faecal samples were from older animals. In fact, this coccidian species is most common in puppies under 6 months of age (Barutzki and Schaper, 2013).

Other parasite stages are to be considered as incidental findings. The present results do not reflect the true prevalence of cestode infection. This is because the sensitivity of faecal flotation, especially when using saline with a specific gravity of 1.2, to detect them is low compared to other methods (adhesive tape method, necropsy, coproantigen test, molecular methods) (Deplazes and Eckert, 1988; Conraths and Deplazes, 2015). Previous necropsy studies showed infections with *Taenia* spp. (*Taenia pisiformis*, *Taenia multiceps*, *Taenia hydatigena*) in 2–8%, *Echinococcus granulosus sensu lato* in 3–13% and *Dipylidium caninum* in 9–34% of free-roaming dogs from western, northern and central Kazakhstan (Lider et al., 2010; Valieva, 2012; Sultanov et al., 2014). The detection of *Trichuris* eggs in a single dog is also a questionable finding because the saline flotation method used is unsuitable for their reliable detection (Deplazes et al., 2016). It should be noted, however, that necropsy

studies in Kazakhstan have also failed to detect *Trichuris* infection in dogs (Lider et al., 2010; Sultanov et al., 2014).

## 5. Conclusions

*Toxascaris leonina* was the dominant intestinal nematode species in free-roaming dogs in Astana, a city known for its large stray dog population. The present results confirm those of other studies in Eurasian regions. It is therefore reasonable to assume that the global prevalence of *T. leonina* infection has been underestimated and should be revised accordingly.

## Ethical statement

The collection of faecal samples and the manipulation of the animals had the approval of the director of the Astana municipal animal shelter and followed local animal welfare standards.

## Author contribution

**Christian Bauer:** Writing – original draft, review & editing, Supervision, Conceptualization. **Lyudmila A. Lider:** Methodology, Investigation, Formal analysis, Data curation. **Altay E. Ussenbayev:** Methodology, Investigation, Formal analysis. **Dinara M. Seitkamzina:** Methodology, Investigation, Formal analysis. **Asylbek A. Zhanabayev:** Methodology, Investigation. **Pavlo Maksimov:** Editing, Software. **Martin Knaus:** Supervision, Conceptualization.

## Declaration of competing interest

The information presented is for scientific purposes only. The authors declared no conflict of interest with respect to the research, authorship and/or publication of this article.

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