# ORIGINAL PAPER

# *Toxocara canis* in household dogs: prevalence, risk factors and owners' attitude towards deworming

R. Nijsse • H. W. Ploeger • J. A. Wagenaar • L. Mughini-Gras

Received: 12 October 2014 / Accepted: 5 November 2014 / Published online: 3 December 2014 © Springer-Verlag Berlin Heidelberg 2014

Abstract The prevalence of gastrointestinal parasites and risk factors for shedding of Toxocara eggs were determined for 916 Dutch household dogs older than 6 months. Additionally, the owners answered a questionnaire about their dogs and their attitude towards routine deworming was assessed. Faecal samples were examined using the centrifugal sedimentation flotation method. The overall prevalence of dogs shedding Toxocara eggs was 4.6 %. Multivariable logistic regression analysis revealed that the risk for 1–7-year-old dogs to shed Toxocara eggs was significantly lower (OR 0.38) than that of 6-12-month-old dogs. Compared to dogs walking  $\leq 20$  % of the time off-leash, those ranging freely 50–80 % and 80-100 % of the time had a significantly higher risk (OR 10.49 and 13.52, respectively) of shedding Toxocara eggs. Other risk factors were coprophagy (OR 2.44) and recently being kenneled (OR 2.76). Although the applied deworming frequency was not significantly associated with shedding Toxocara eggs, there was a trend towards no shedding in dogs under strict supervision that were dewormed 3-4 times a year. Most dog owners (68 %) recognized 'dog's health' as the main reason for deworming. Only 16 % of dogs were dewormed four times a year. It was concluded that the prevalence of Toxocara egg-shedding household dogs is almost unchanged over recent years and

J. A. Wagenaar

Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands

that the knowledge of owners is insufficient to expect sound decisions on routine deworming.

**Keywords** Deworming frequency · Gastrointestinal parasites · Faecal samples · *Toxocara canis · Toxocara* eggs · Dog

## Introduction

Toxocara canis rarely causes disease in adult dogs, and for this reason, it does not warrant treatment. However, it is a parasite with zoonotic potential, as it may cause visceral and ocular larva migrans and allergic airway inflammation in humans (Pinelli et al. 2008; Pinelli and Aranzamedi 2012). Therefore, the guidelines of the European Scientific Counsel Companion Animal Parasites (ESCCAP) state that all adult dogs should be dewormed at least four times a year to prevent patent T. canis infections in dogs. In situations where there is a high risk of human exposure to Toxocara eggs, the advice is to deworm dogs up to 10-12 times a year (ESCCAP 2010). However, several cross-sectional surveys indicate that well over 90 % of all adult household dogs do not shed Toxocara eggs (Overgaauw 1997b; Claerebout et al. 2009; Overgaauw et al. 2009). This implies that many dogs are treated while they have no adult worms in their intestines. This does not conform to the principle of good veterinary practice (GVP) promoting the use of medicines only when required and following a diagnosis (Federation of Veterinarians of Europe, 2002), even though routine preventative anti-parasitic treatments of companion animals have been defined as an exception to the principles of GVP. Furthermore, there is no evidence that treating dogs every 3 months prevents patent Toxocara infections (Sager et al. 2006; Claerebout et al. 2009). T. canis has a prepatent period of slightly over 1 month after ingestion of infective eggs, leaving ample time for susceptible dogs to

R. Nijsse (⊠) · H. W. Ploeger · J. A. Wagenaar · L. Mughini-Gras Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, Postbus 80163, 3508 TD Utrecht, The Netherlands e-mail: E.R.Nijsse@uu.nl

L. Mughini-Gras

National Institute for Public Health and the Environment, Centre for Infectious Disease Control (CIb), Bilthoven, The Netherlands

acquire a patent infection between successive moments of treatment. The prepatent period can be even shorter when an infection is obtained by ingesting a paratenic host, as no hepatic-tracheal migration would be necessary for the larvae to develop into adult worms (Warren 1969). Therefore, guidelines should either unequivocally advocate 11-12 treatments per year (based on the prepatent period of T. canis) or they should focus on targeted treatments considering specific risk factors and involving faecal examinations. Current deworming guidelines are not mandatory to apply, and achieving a high compliance is notoriously difficult (Anonymous 2003; Overgaauw and Boersema 1996; Overgaauw et al. 2009). It can therefore be questioned whether any effort aimed at increasing the deworming frequency to 11-12 times a year for all dogs is worthwhile rather than, e.g. promoting targeted treatments based on the actual risk for a dog to be a shedder of Toxocara eggs. It is crucial to examine risk factors for shedding Toxocara eggs in dogs, including owners' knowledge, attitudes and practices towards Toxocara control measures, to provide an evidence base for implementing targeted deworming strategies over the advocated blind treatments for all dogs.

Previous studies identified several risk factors for patent Toxocara infections, although not unequivocally. For instance, in a large study comprising 1.2 million dogs in the United States (US), dog's age, body weight, sex, breed and geographic origin were associated with intestinal nematode parasitism, including T. canis (Mohamed et al. 2009). Dog's age and household income were strong predictors of patent infections in another US study (Gates and Nolan 2009). A Finnish study identified being kenneled and foreign travel as risk factors for T. canis and Uncinaria stenocephala infections in dogs (Pullola et al. 2006). Among Swiss household dogs, eating offal, carrion or garbage were risk factors for shedding Toxocara eggs (Sager et al. 2006). Among Polish sled dogs, sex was not significantly associated with the prevalence of intestinal parasites, but residing in a large kennel and being <2 years or >8 years of age were significant risk factors for T. canis infection (Bajer et al. 2011). Finally, in a Belgian study, only in kenneled, but not in household, dogs a significant association between age and T. canis infection was found (Claerebout et al. 2009). The same study showed that a high number of anthelmintic treatments in household dogs was associated with a higher T. canis prevalence. Comparing these studies is difficult due to their different designs, dog populations and definitions of outcome and exposure. Other influencing factors, such as coprophagy (Fahrion et al. 2011, Nijsse et al. 2014), as well as clustering effects due to dogs living in groups (e.g. in the same household, kennel, etc.) can distort or confound the actual exposure egg-shedding relations.

Apart from identifying risk factors, it is important to assess the decisive reason(s) for owners to deworm their dog(s). This, combined with the compliance with the advocated deworming regimens, can provide insights in the driving factors behind the decisions that owners make about deworming their dogs.

The aims of this study were to (1) determine the coprological prevalence of *Toxocara* eggs, among those of other helminths, in Dutch household dogs older than 6 months, not linked to a shelter or veterinary clinic, (2) define the relation between the reported deworming frequency and prevalence of patent *Toxocara* infections as well as risk factors for shedding *Toxocara* eggs, and (3) assess whether there is an association between owners' reasons for deworming and the application of specific deworming regimens, and whether these reasons are significant predictors of shedding *Toxocara* eggs by dogs.

# Material and methods

## Participants and questionnaire

Between July 2011 and August 2012, 566 dog owners voluntarily submitted a faecal sample of their dog(s) for coproscopical examination for parasite eggs and (oo)cysts to the Faculty of Veterinary Medicine of Utrecht University and completed a web-based self-administered questionnaire to collect relevant epidemiological information.

The possibility to participate in the study was publicized in pet shops, veterinary clinics, pet-themed websites and dog breed societies in the Netherlands. Additionally, flyers were handed out at dog walking areas. Dogs were required to be at least 6 months old and, for logistic reasons, each owner was allowed to submit faeces of a maximum of four dogs.

Results of the coproscopical examination were communicated to the owner after completion of the questionnaire. The questionnaire was in Dutch and contained questions concerning the dog's age, sex, breed, function, reproductive status, living conditions, diet, time roaming freely, predatory and coprophagic behaviour, health status, medication use and deworming history. A section about the application of anthelmintics by the owner (i.e. reason for deworming their dog(s) and the applied deworming frequency) was included in the questionnaire. A copy of the questionnaire is available on request to the authors. In total, a faecal sample and the corresponding questionnaire were available for 916 dogs.

# Coproscopical examination

Faecal samples were identified individually. Instructions and materials to collect and send the faecal sample to the laboratory were provided to the owners. Faecal suspensions consisting of 3 g of faeces and 55 ml of water were examined using the centrifugal sedimentation flotation method with sucrose as flotation solution (s.g.  $1.27-1.30 \text{ g/cm}^3$ ). For logistical reasons, faecal samples were first pooled including two samples per test tube at a time and then re-tested individually in cases of any positivity. Centrifugation took place at 3,000 rpm (Rotofix 32, Hettich zentrifugen) for 2 min for both sedimentation and flotation. Centrifugation for flotation took place with the cover slide on top of the test tube. Diagnosis, based on morphometric characteristics, of parasite eggs and (oo)cysts in the faeces was performed using light microscopy at magnification  $100-400\times$ .

## Data analysis

Differences in proportions were assessed using the  $\chi^2$  test or the Fisher's exact test, as appropriate. For preliminary significance testing, we assessed univariately the association of 32 variables with positivity for Toxocara eggs using unconditional logistic regression. The potential confounders dog's age (categorized as 6 months–1 year, 1-7 years and >7 years, according to pet food industry standard categorization for respectively young, adult and mature dogs) and reported coprophagic behaviour were controlled for by always including them as covariates in the models. Variables showing a pvalue lower than 0.25 for the association with the outcome variable in the single-variable analysis were selected for inclusion in a multivariable logistic regression model. A backward stepwise selection procedure was applied, and variables with a p value lower than 0.05 were retained in the final model. Variables were dropped one by one starting from the least non-significant one and then adding back all dropped variables if they later appeared to be significant when re-added in the reduced model. This procedure did not, however, lead to new significant associations. Also, the effect of removing and adding variables on the associations of the other variables included in the model was monitored. A change of  $\geq 10$  % in the regression coefficients was considered as a sign of confounding, so the variable was retained into the model regardless of its significance. Associations were expressed as odds ratios (ORs) with 95 % confidence intervals (95 % CI). This did not lead to a new assembly of variables. All models accounted for non-independency in the data due to clustering of dogs living in the same household using a cluster-correlated robust variance estimator (Williams 2000). Subsequently, first-order interactions were tested between all included significant variables. However, no interaction was significant, so the final model was not expanded to include significant interaction terms. The final multivariable model showed an overall statistical significance (likelihood ratio  $\chi^2$  test, p < 0.05) and goodness-of-fit (Hosmer-Lemeshow test, p > 0.05). Statistical analysis was performed using STATA 11 (StataCorp LP, College Station, USA).

#### Results

Prevalence of gastrointestinal parasites

Of the 916 faecal samples examined, 74 were found positive for at least one type of helminth egg (8.1 %, 95 % CI: 6.4– 10.1 %). In 68 dogs, only one type of egg was found; four dogs showed two types of eggs, and two dogs had a triple infection. The most frequently found egg type was that of *Toxocara* sp. The different types of helminth eggs that were recovered are shown in Table 1.

# Risk factors

As the main focus of this study was on *T. canis*, risk factors were defined for this specific parasite only.

Coprological prevalence of *Toxocara* eggs was significantly different among age groups (p < 0.05). Dogs aged between 6 months and 1 year (n=230) showed the highest prevalence (7.8 %, 95 % CI: 4.7–12.1 %), followed by those aged >7 years (4.0 %, 95 % CI: 1.8–7.8 %; n=198) and by those between 1 and 7 years of age (3.3 %, 95 % CI: 1.9–5.3 %; n=488). The majority of examined dogs (n=521, 56.9 %) was female, nine (1.7 %) of which were pregnant at the time of sampling, but no significant difference in the presence of *Toxocara* eggs was found between faeces of male and female dogs nor between those of pregnant and non-pregnant dogs.

Dogs displaying coprophagic behaviour according to their owner (n=399, 43.6 %) had a significantly higher (p<0.05) faecal prevalence of *Toxocara* eggs (7.3 %, 95 % CI: 4.9–10.3 %) compared to those dogs (n=517) for which the respective owners did not report such behaviour (2.5 %, 95 % CI: 1.4–4.3 %).

The living environment of the dogs was reported by the owners based on the prevalent characteristics of their neighbourhood as suggested by the questionnaire; an urban/ residential area was defined as the one containing mainly paved roads, sidewalks and houses with small or no green areas; a rural area contained few trees but mainly pastures and meadows; and a woody areas consisted mainly of forests and shrubs. There were no significant differences in the coprological

 Table 1
 Helminth egg types recovered after coproscopical examination of 916 household dogs

Helminths	п	Prevalence	95 % CI
Toxocara sp.	42	4.6 %	3.3-6.2 %
Hookworms	19	2.1 %	1.3-3.2 %
Trichuris sp.	9	1.0 %	0.5-1.9 %
<i>Capillaria</i> sp.	8	0.9 %	0.4–1.7 %
Taeniidae	3	0.3 %	0.1-1.0 %
Toxascaris leonina	1	0.1 %	0.0-0.6 %

prevalence of *Toxocara* eggs among dogs living in urban/ residential (3.7 %, 95 % CI: 2.3–5.8 %; n=508), rural (5.0 %, 95 % CI: 2.2–9.7; n=159), woody (8.2 %, 95 % CI: 2.7–18.1; n=61) or mixed (5.3 %, 95 % CI: 2.6–9.6; n=188) environments. No significant differences in the coprological prevalence of *Toxocara* eggs were detected among seasons (summer, June-August: 3.4 %, 95 % CI: 2.1–5.2, n=610; spring, March-May: 4.3 %, 95 % CI: 1.2–10.8, n=92; autumn, September-November: 8.1 %, 95 % CI: 4.4–13.4, n=161; winter, December-February: 7.5 %, 95 % CI: 2.1–18.2, n=53).

Dogs that were kenneled, i.e. and temporarily placed out of their homes at least once in the 2 months prior to sampling, tested positive for *Toxocara* eggs significantly more often (p<0.05) than dogs that were not kenneled (9.6 %, 95 % CI: 3.9–18.8, n=73 vs. 4.2 %, 95 % CI: 2.9–5.8, n=839). For four dogs, this information was missing.

The percentage of walking time during which the dogs could range freely (i.e. off-leash and/or unsupervised by their owners) had a significant effect (p < 0.05) on the coprological prevalence of *Toxocara* eggs. Dogs wandering 81–100 % of their walking time freely showed the highest prevalence (6.4 %, 95 % CI: 3.8–10.0 %, n=266), followed by dogs ranging freely for 51–80 % (6.0 %, 95 % CI: 3.5–9.5, n=268), 21–50 % (3.7 %, 95 % CI: 1.6–7.2, n=214) or  $\leq 20$  % (0.6 %, 95 % CI: 0.0–3.3, n=165) of their walking time.

Predation was not significantly associated with shedding of *Toxocara* eggs. Prevalence of *Toxocara* eggs in predating dogs was 3.6 % (95 % CI: 1.2–8.3, n=137), in non-predating dogs 4.7 % (95 % CI: 3.1–6.8, n=557) and in dogs with unknown history of predation 5.0 % (95 % CI: 2.5–8.7, n=222). This was true also when considering the reported actual consumption of the prey.

Of all dogs, 99 (10.8 %) never received an anthelminthic treatment according to the owner, 197 (21.5 %) were treated at least once a year, 177 (19.3 %) twice a year, 106 (11.6 %) three times a year and 148 (16.2 %) four or more times a year. Of the remaining dogs, 117 (12.8 %) were treated upon some form of indication (e.g. by the veterinary practitioner following coprological examination, before vaccinations, travelling abroad, etc.), when the dog showed any symptom that could be associated with a helminth infection (e.g. diarrhoea, weight loss, perineal itching, visible presence of worms in faeces, etc.) or when there was any other reason to think that the dog could have been infected (e.g. travel, stay in kennel/shelter, ingestion of faeces, dirty water, dead animals, etc.). For 72 dogs (7.86 %), the history of anthelminthic treatment was unknown. The frequency of treatment did not have a significant effect on the prevalence of Toxocara eggs in dog faeces (Table 2). After deleting those dogs that displayed coprophagic behaviour, that were kenneled in the 2 months prior to sampling, and that could walk freely more than 50 % of their time, no coprological positivities for Toxocara eggs were demonstrable in dogs dewormed three to four times a year, although these differences remained not statistically significant (NS).

Of the examined dogs, 100 (10.9 %) had received an anthelminthic treatment within 1 month before sampling, 75 (8.2 %) between 1 and 2 months, 100 (10.9 %) between 2 to 3 months and 484 (52.8 %) more than 3 months before. For 157 (17.1 %) dogs, this information was unknown. The timing of last deworming did not have a significant effect on the prevalence of *Toxocara* eggs in dog faeces (Table 3). After removing dogs that displayed a coprophagic behaviour, that were kenneled in the 2 months prior to sampling, and that could walk freely more than 50 % of their time, no coprological positivities to *Toxocara* eggs were demonstrable in dogs dewormed within 2 months from sampling, although these differences remained statistically NS.

In the single-variable logistic regression analysis, after adjusting for dog's age and coprophagy, as well as accounting for clustering of dogs living in the same household, eight variables with a p value  $\leq 0.25$  for the association with the presence of Toxocara eggs in dog's faeces were selected for inclusion in the multivariable logistic regression model (Table 4). In the final multivariable model, only two of these variables in addition to age and coprophagy remained significant. Dogs that stayed in a kennel in the last two months prior to sampling had a 2.76 times significantly higher risk of being *Toxocara*-positive than dogs that were not kenneled (p < 0.05). Compared to dogs ranging freely for  $\leq 20$  % of their walking time, the risk of being Toxocara-positive for dogs that could walk off-leash for 51-80 % and 81-100 % of their time was 10.49 (p < 0.05) and 13.52 (p < 0.05) times higher, respectively. Also, dogs that were allowed to walk off-leash for 21-50 % of their time had, on average, a 6.51 times higher risk of being Toxocara-positive compared to the dogs walking off-leash  $\leq 20$  % of their time, but this difference was NS. Compared to young dogs between 6 months and <1 year of age, dogs aged 1-7 years had a 0.38 times lower risk of being Toxocarapositive (p < 0.05), while older dogs (>7 years of age) still had, on average, a 0.46 times lower risk of being Toxocara-positive than puppies (NS). Dogs showing a coprophagic behaviour had a 2.44 significantly higher risk of having Toxocara eggs in their faeces compared to those dogs for which their owner did not report such behaviour (p < 0.05).

# Owner's perception towards deworming

Information about the owner's main reason for anthelmintic treatment of their dogs was answered by 497 owners and available for 801 dogs. Not every owner answered this section of questions for all of their dogs, and not every owner was consistent in applying the same deworming regime for all the dogs in the same household. 'The dog's health' was the main reason for 336 owners (68 %) to deworm 534 dogs (67 %). 'Public health' was recognized by 72 (14 %) owners as the

Deworming frequency	All dogs			Non-coprophagic, unkenneled and leashed dogs <sup>b</sup>			
	n	<i>Toxocara</i> -positive dogs	<i>Toxocara</i> prevalence (95 % CI)	n	<i>Toxocara</i> -positive dogs	<i>Toxocara</i> prevalence (95 % CI)	
Never	99	3	3.0 (0.6-8.6)	26	1	3.8 (0.1–19.6)	
Once a year	197	7	3.6 (1.4–7.2)	43	1	2.3 (0.1–12.3)	
Twice a year	177	6	3.4 (1.3–7.2)	39	2	5.1 (0.7–17.3)	
Three times a year	106	7	6.6 (2.7–13.1)	24	0	0.0 (0.0–14.2) <sup>a</sup>	
Four times a year	148	12	8.1 (4.3–13.7)	36	0	0.0 (0.0–9.7) <sup>a</sup>	
On indication	117	4	3.4 (0.9-8.5)	36	1	2.8 (0.1–14.5)	
Unknown	72	3	4.2 (0.9–11.7)	15	0	0.0 (0.0–21.8) <sup>a</sup>	
Total	916	42	4.6 (3.3–6.2)	219	5	2.3 (0.7–5.3)	

 Table 2
 Frequencies of Toxocara-positive dogs under different deworming regimens in the whole dog population and in non-coprophagic, unkenneled and leashed dogs

<sup>a</sup> One-sided, 97.5 % confidence interval

<sup>b</sup> Leashed dogs are defined as dogs wandering off-leash less than 50 % of their walking time

most important reason for deworming 111 (14 %) dogs. The option 'because we must' was answered for 57 (7 %) dogs by 32 (6 %) owners. The combination public health and the dog's health was the reason that 34 (7 %) owners dewormed their 54 (7 %) dogs. 'Another reason' was answered by 23 owners; 69 owners did not answer this question.

After these data were cross-tabulated against the applied deworming frequency, and dogs that were not dewormed and owners answering another reason were discarded, 597 dogs remained (Table 5). There was no significant association between the main reason for deworming and the applied deworming frequency.

## Discussion

The need of changing the current approach towards deworming in household dogs is indicated by several studies conducted in the Netherlands and bordering countries that indicate similar prevalences of household dogs shedding *Toxocara* eggs over almost two decades. For instance, in 1997, 2.9 % of faecal samples from household dogs tested positive for *Toxocara* eggs (Overgaauw 1997b), 4.6 % in 2007 (Overgaauw et al. 2009), 4.4 % between 2004 and 2007 (Claerebout et al. 2009) and 4 % in 2011 (Becker et al. 2012). Although the effect of the ESCCAP deworming recommendations introduced in 2006, which advise to deworm twice as often compared to the old regimen, are thought to have led to a lower seroprevalence of *Toxocara* infection in humans (Pinelli et al. 2011), this is not reflected in the *Toxocara* shedding prevalence among dogs.

Younger age proved, as expected, to be an independent risk factor for canine toxocariasis, even though the minimum age of the participating dogs was 6 months. This indicates that the described age resistance to *Toxocara* infection (Ehrenford 1957; Greve 1971) is not absolute. Besides age, the main risk factors identified in this study were essentially those related to

 Table 3
 Frequencies of Toxocara-positive dogs according to time since last deworming in the whole dog population and in non-coprophagic, unkenneled, and leashed dogs

Time elapsed since last deworming	All dogs			Non-coprophagic, unkenneled and leashed dogs <sup>b</sup>			
	n	<i>Toxocara</i> -positive dogs	<i>Toxocara</i> prevalence (95 % CI)	n	<i>Toxocara</i> -positive dogs	<i>Toxocara</i> prevalence (95 % CI)	
≤1 month	100	8	8.0 (3.5–15.2)	21	0	0.0 (0.0–16.1) <sup>a</sup>	
1–2 months	75	1	1.3 (0.0–7.2)	23	0	0.0 (0.0–14.8) <sup>a</sup>	
2–3 months	100	6	6.0 (2.2–12.6)	18	1	5.6 (0.1-27.3)	
>3 months	484	21	4.3 (2.7–6.6)	118	3	2.5 (0.5–7.3)	
Unknown	157	6	3.8 (1.4-8.1)	39	1	2.6 (0.1–13.5)	
Total	916	42	4.6 (3.3–6.2)	219	5	2.3 (0.7–5.3)	

<sup>a</sup> One-sided, 97.5 % confidence interval

<sup>b</sup> Leashed dogs are defined as dogs wandering off-leash less than 50 % of their walking time

Variable	% exposed among <i>Toxocara</i> - positive dogs ( <i>n</i> =42)	% exposed among <i>Toxocara</i> - negative dogs ( <i>n</i> =874)	Single-variable OR <sup>a</sup> (95 % CI)	Multivariable OR <sup>b</sup> (95 % CI)	
Age group					
<1 year	42.86	24.26	Ref.	Ref.	
1–7 years	38.10	54.00	0.44 (0.21-0.91)	0.38 (0.18-0.80)	
>7 years	19.05	21.74	0.50 (0.20-1.23)	0.46 (0.19–1.12)	
Coprophagy	69.05	42.33	2.83 (1.29-6.16)	2.44 (1.14–5.18)	
% time the dog wanders unsupervised					
0–20 %	2.38	18.76	Ref.	Ref.	
21-50 %	19.05	23.57	5.87 (0.70-49.34)	6.51 (0.75–56.89)	
51-80 %	30.10	28.84	8.99 (1.19-69.30)	10.49 (1.30-84.43)	
81–100 %	40.48	28.49	11.42 (1.48-88.03)	13.52 (1.65–110.54)	
Being a farm dog	4.76	1.72	2.81 (0.59–13.33)		
Kenneled in the last 2 months	16.67	7.55	2.10 (0.81-5.45)	2.76 (1.06-7.17)	
Owning pet birds	4.76	11.33	0.38 (0.09–1.55)		
Owning pet rabbits	4.76	13.27	0.37 (0.09–1.57)		
Feeding the dog with a commercial diet	73.81	59.27	1.97 (0.93-4.16)		
Feeding the dog with frozen raw meat	33.33	51.60	0.47 (0.23-0.95)		
Medicated in the last 3 months <sup>c</sup>	2.38	9.84	0.21 (0.03–1.57)		

<sup>a</sup> Adjusted for age, coprophagy, except for the eponymous variables, and clustering of dogs living in the same households

<sup>b</sup> Adjusted for age, coprophagy, except for the eponymous variables, clustering of dogs living in the same households, and for the other variables included in the model

<sup>c</sup> Excluding dietary supplements (e.g. vitamins, minerals, etc.) and homeopathic compounds

Deworming frequency per year		Reason for deworming						
		Dog's health	Public health	Combination <sup>b</sup>	Dogmatic <sup>c</sup>	Total		
1×	No	118	29	8	20	175		
	Row%	67.4	16.6	4.6	11.4	100		
	Column%	28.1	31.9	19.1	45.5	29.3		
2×	No	123	27	16	9	175		
	Row%	70.3	15.4	9.1	5.1	100		
	Column%	29.3	29.7	38.1	20.5	29.3		
3×	No	79	9	7	7	102		
	Row%	77.5	8.8	6.9	6.9	100		
	Column%	18.8	9.9	16.7	15.9	17.1		
4×	No	100	26 <sup>a</sup>	11	8	145		
	Row%	69.0	17.9 <sup>a</sup>	7.6	5.5	100		
	Column%	23.8	28.6 <sup>a</sup>	26.2	18.2	24.3		
Total	No	420	91	42	44	597		
	Row%	70.4	15.2	7.0	7.4	100		
	Column%	100	100	100	100	100		

Table 5 Reasons for deworming and applied deworming frequencies in individual dogs

<sup>a</sup> ESCCAP advised guidelines for standard blind deworming

<sup>b</sup> Reason for deworming was a combination of 'dog's health' and 'public health'

<sup>c</sup> The recognized reason for deworming by the owner was 'because we must'

an owner's loss of control over the respective dog, e.g. when a dog is free-roaming for more than half of its walking time or when a dog is being cared for out-of-home in a kennel. This way, dogs are able to ingest (contaminated) materials from the environment relatively unnoticed, somehow resembling stray dogs in which a higher Toxocara prevalence is to be expected (le Nobel et al. 2004; Becker et al. 2012). Predation is also recognized as a cause of patent infection in adult dogs (Warren 1969; Overgaauw 1997a; Sager et al. 2006; Strube et al. 2013). Toxocara larvae ingested from paratenic hosts can mature in the dog's intestine without completing the tracheal migration and thus evade the dog's immunity/age resistance. Predation was not, however, identified as a significant risk factor in our study. Predatory behaviour is not necessarily a risk factor per se; therefore, we also assessed the association with the actual consumption of the prey. Although there was a positive association between positivity for Toxocara and consumption of prey animals, this was NS, presumably due to the small number of owners reporting the actual consumption of the prey by their dogs (data not shown). Follow-up studies comparing predating and not predating dogs for a longer period are needed to capture the risk for Toxocara infection posed by predation.

The same holds for other factors that were not significantly associated with shedding of *Toxocara* eggs, such as feeding of raw meat. The lack of significance of this association is likely to be due to the unknown origin of the meat the dog was fed with. To pose a risk of infection, the meat needs to contain viable *Toxocara* larvae. Slaughter animals, therefore, need to have ingested embryonated eggs from their environment. However, most of the meat sold in the Netherlands comes from intensive animal husbandry in which infection of the animals with *T. canis* eggs will be unlikely.

No significant correlation was found between the shedding of Toxocara eggs and a dog's living environment. Yet, dogs living in urban areas showed the lowest prevalence (3.7 % vs.)at least 5.0 to 8.2 % in other areas). As the living environment might not be the same as where the dogs are actually walked, this finding is hard to interpret even more considering the fact that infections with larvated Toxocara eggs usually do not result in patent infections in adult dogs. The number of eggs and the immune response of the dog complicate the interpretation of the association of mere environmental contamination and availability of eggs and patent Toxocara infections (Dubey 1978; Glickman et al. 1981; Fahrion et al. 2008). Red foxes are common in the Netherlands, and a rural or woody living environment with a relatively low density of dogs can be equally contaminated as an urban area with a high dog density due to the contribution of foxes shedding Toxocara eggs in a relatively high prevalence (Borgsteede 1984; Franssen et al. 2014).

Toxocara eggs present in the environment may be either embryonated or not. While ingestion of unembryonated eggs will not lead to an infection, eggs containing infective larvae may lead to a patent infection depending on the age and immunological status of the dog. It is important for epidemiological studies to differentiate patent infections from passive passage of unembryonated Toxocara eggs. This is supported by this present study, as coprophagy was a significant risk factor for dogs shedding Toxocara eggs. Finding these eggs in dogs' faeces does, therefore, not necessarily mean that these dogs have a patent infection as unembryonated Toxocara eggs are able to pass the gastrointestinal tract seemingly unaffected (Fahrion et al. 2011; Nijsse et al. 2014). Coprophagy alone did not suffice in explaining those dogs that tested positive for Toxocara eggs within 1 month from the last deworming (data not shown), which is within the prepatent period. An additional explanation could be that the deworming itself was not successful because the dog did not ingest a tablet or spot-on products were not applied lege artis. Anthelminthic resistance in dog helminths is not yet found in the Netherlands and also might not be expected as the refugia is large due to a high number of owners who do not deworm their dogs intensively and the high prevalence of infection in the red fox population (Borgsteede 1984; Franssen et al. 2014).

The applied deworming frequency reported by the owners showed no significant association with positivity for Toxocara eggs at coproscopical examination when the entire study population was included. This can be expected when the period after the duration of the effect of the last deworming exceeds the prepatent period. This is in line with results from other studies (Sager et al. 2006; Claerebout et al. 2009). However, the shedding of Toxocara eggs appears to be prevented when dogs are treated at least three times a year when coprophagic dogs, recently kenneled dogs and dogs that are walking off-leash more than 50 % of the time were excluded. This suggests that the ESCCAP advised deworming regimen may be able to prevent shedding of Toxocara eggs in dogs in the low-risk categories, i.e. in dogs that were not kenneled recently, that did not walk off-leash most of the time and that did not show an evident coprophagic behaviour. It is not clear whether the observed effect is indeed due to the treatment. However, if it were solely due to the removal of the dogs exposed to the above-mentioned three risk factors, one might have expected no Toxocara eggs in dogs treated less frequently as well. Because of the very small numbers of positive samples in the remainder of our dog population after removing those dogs that were at high risk, no definitive conclusion can be drawn, although there is some suggestive evidence that deworming 3-4 times a year prevents dogs from shedding Toxocara eggs, at least in low-risk dogs.

A suboptimal compliance by owners to the proclaimed deworming advice in the Netherlands (Overgaauw and Boersema 1996; Overgaauw et al. 2009) and outside Europe (Lee et al. 2010; Palmer et al. 2010) has been reported. Our study shows a discrepancy between the advocated deworming advice and the reason for implementing this advice by dog owners. The public health concern related to the zoonotic potential of *T. canis* is the driving factor behind the advised four times a year blind deworming regimen. Yet, the majority of owners reported that the main reason for deworming their (young to adult) dogs blindly was the dog's health. *T. canis*, however, mainly causes disease in puppies and generally not in adult, well-cared dogs. If the dog's health is the main reason for deworming, an owner of a dog without clinical symptoms is not intrinsically motivated to deworm. This may provide an explanation for the generally low compliance to the advised deworming regimen.

This study has some limitations. Participation on a voluntary basis could have led to some selection bias, especially regarding the owners' attitudes towards deworming. These owners might have well consisted of a self-selected group of particularly motivated people with special fondness for their dogs' health, being also willing to enrol voluntarily to the study, collect and send in a faecal sample of their dogs, invest time in answering a questionnaire and replicate all these steps for each dog they owned. However, because of the variety of answers provided to the question about the applied deworming regimens, the selection of participants is not expected to have biased our results significantly. Moreover, reported behaviours of dogs need to be interpreted with caution, as owners do not always (want to) see unpleasant behaviours (e.g. coprophagy) in their dogs.

In areas where, for example, *Dirofilaria immitis* is endemic owners are usually aware of the health risk for their dogs and, therefore, may comply more with the advised deworming regimen. Our results indicate that education of dog owners about the public health hazards posed by *T. canis*, whose infection is not necessarily associated with symptoms in their dogs, needs more attention. The majority of dog owners (still) do not recognize the public health issue surrounding *Toxocara* as the most important factor for deworming. Responsible dog ownership concerning dog's health and public health should be better propagated by veterinarians, pet shops and breeders even though the actual burden of illness due to toxocariosis among people is unclear.

In this study, as expected, about 95 % of dogs were not shedding *Toxocara* eggs. This information is not an incentive for owners to comply with the advised blind deworming regimen. Conversely, identifying dogs that are at high risk of shedding *Toxocara* eggs is more likely to convince owners of a need to treat. The risk factors identified here may in fact be translated to risk-based deworming advices for owners. This applies to young dogs (<1 year), dogs roaming freely more than half of their walking time and dogs that are being kenneled or have been kenneled recently. These advices may include additional faecal examinations, extra deworming

treatments and the explicit advice and strict enforcement of cleaning-up policies for dog faeces.

## Conclusion

The observed prevalence of 4.6 % of dogs shedding *Toxocara* eggs is in agreement with previous studies on household dogs. Young age, coprophagy, recent stay in a kennel and free-ranging more than half of the walking time were identified as independent risk factors for shedding of *Toxocara* eggs.

Only 24 % of the dogs were treated by their owners in agreement with ESCCAP recommendations (i.e. four times a year, blindly) and only 18 % of these dogs because of public health concerns. As this reason is not recognized as the most important one, better compliance with the recommended deworming schedule may require a significant improvement in effectively informing owners on why they should treat their dogs.

The applied deworming schedule is not associated with the actual shedding of Toxocara eggs. When dogs at high risk of shedding Toxocara eggs (i.e. coprophagic, previously kenneled and predominantly free-ranging dogs) were accounted for, no dog shedding Toxocara eggs was present among those dewormed 3-4 times a year, but given the low numbers, this could not be proven statistically. This also applied to the time elapsed between sampling and last deworming. Although definitive conclusions cannot be drawn, it seems that there is a trend towards no shedding of Toxocara eggs in dogs under strict supervision by owners when these were dewormed 3-4 times a year. For dogs at high risk of shedding Toxocara eggs, more frequent faecal examinations, when proven necessary additional deworming treatments and strict enforcement of cleaning-up of dog faeces seem to be the most recommendable means for reducing the environmental contamination with Toxocara eggs by household dogs.

Acknowledgments The authors thank Bayer Animal Health for partially financing this study. Bayer Animal Health did not have any influence on the design of this study or on the outcomes. The Veterinary Microbiological Diagnostic Centre of the Faculty of Veterinary Medicine of Utrecht University, Stephanie Rodenberg and students performing their research internship are acknowledged for their substantial contribution to the laboratory work and Nicole Buijtendijk for the administrative support. The owners participating in this study are acknowledged for supplying faeces and answering the questionnaire.

**Conflicts of interest** The authors of this paper state that there are no conflicts of interest concerning this paper.

Bayer Animal Health had no saying in the study design nor in the outcome or reporting of the results.

Though the corresponding author is involved in the ESCCAP organization as the secretary of ESCCAP Benelux, this did not influence the study design nor the outcome or reporting of the results.

#### References

- Anonymous (2003) AAHA study finds millions of pets aren't getting maximum health care. J Am Vet Med Assoc 222:1488
- Bajer A, Bednarska M, Rodo A (2011) Risk factors and control of intestinal parasite infections in sled dogs in Poland. Vet Parasitol 175:343–350
- Becker AC, Rohen M, Epe C, Schnieder T (2012) Prevalence of endoparasites in stray and fostered dogs and cats in Northern Germany. Parasitol Res 111:849–857
- Borgsteede FH (1984) Helminth parasites of wild foxes (*Vulpes vulpes* L.) in The Netherlands. Z Parasitenkd 70:281–285
- Claerebout E, Casaert S, Dalemans AC, De Wilde N, Levecke B, Vercruysse J, Geurden T (2009) *Giardia* and other intestinal parasites in different dog populations in Northern Belgium. Vet Parasitol 161:41–46
- Dubey JP (1978) Patent *Toxocara canis* infection in ascarid-naive dogs. J Parasitol 64:1021–1023
- Ehrenford FA (1957) Canine ascariasis as a potential source of visceral larva migrans. Am J Trop Med Hyg 6:166–170
- ESCCAP (2010) September 2010-last update. Guideline 01 2nd edition. Worm control in dogs and cats. Available: http://www.esccap.org/ uploads/docs/nkzqxmxn\_esccapgl1endoguidelines.pdf
- Fahrion AS, Staebler S, Deplazes P (2008) Patent Toxocara canis infections in previously exposed and in helminth-free dogs after infection with low numbers of embryonated eggs. Vet Parasitol 177:186–189
- Fahrion AS, Schnyder M, Wichert B, Deplazes P (2011) *Toxocara* eggs shed by dogs and cats and their molecular and morphometric species-specific identification: is the finding of *T. cati* eggs shed by dogs of epidemiological relevance? Vet Parasitol 152:108–115
- Federation of Veterinarians of Europe (2002) Accessed http://www.fve. org/news/publications/pdf/gvp.pdf on July 2014
- Franssen F, Nijsse R, Mulder J, Cremers H, Dam C, Takumi K, van der Giessen J (2014) Increase in number of helminth species from Dutch red foxes over a 35-year period. Parasit Vectors. doi:10.1186/1756-3305-7-166
- Gates MC, Nolan TJ (2009) Risk factors for endoparasitism in dogs: retrospective case-control study of 6578 veterinary teaching hospital cases. J Small Anim Pract 50:636–640
- Glickman LT, Dubey JP, Winslow LJ (1981) Serological response of ascarid-free dogs to *Toxocara canis* infection. Parasitology 82: 383–387
- Greve JH (1971) Age resistance to *Toxocara canis* in ascarid-free dogs. Am J Vet Res 32:1185–1192
- Le Nobel WE, Robben SR, Dopfer D, Hendrikx WM, Boersema JH, Fransen F, Eysker M (2004) Infections with endoparasites in dogs in Dutch animal shelters. Tijdschr Diergeneeskd 129:40–44

- Lee AC, Schantz PM, Kazacos KR, Montgomery SP, Bowman DD (2010) Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. Trends Parasitol 26:155–161
- Mohamed AS, Moore GE, Glickman LT (2009) Prevalence of intestinal nematode parasitism among pet dogs in the United States (2003– 2006). J Am Vet Med Assoc 234:631–637
- Nijsse R, Mughini-Gras L, Wagenaar JA, Ploeger HW (2014) Coprophagy in dogs interferes in the diagnosis of parasitic infections by faecal examination. Vet Parasitol 204:304–309
- Overgaauw PA (1997a) Aspects of *Toxocara* epidemiology: toxocarosis in dogs and cats. Crit Rev Microbiol 23:233–251
- Overgaauw PA (1997b) Prevalence of intestinal nematodes of dogs and cats in The Netherlands. Vet Q 19:14–17
- Overgaauw PA, Boersema JH (1996) Assessment of an educational campaign by practicing veterinarians in The Netherlands on human and animal *Toxocara* infections. Tijdschr Diergeneeskd 121:615–618
- Overgaauw PA, van Zutphen L, Hoek D, Yaya FO, Roelfsema J, Pinelli E, van Knapen F, Kortbeek LM (2009) Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. Vet Parasitol 163:115–122
- Palmer CS, Robertson ID, Traub RJ, Rees R, Thompson RC (2010) Intestinal parasites of dogs and cats in Australia: the veterinarian's perspective and pet owner awareness. Vet J 183:358–361
- Pinelli E, Aranzamedi C (2012) *Toxocara* infection and its association with allergic manifestations. Endocr Metab Immune Disord Drug Targets 12:33–44
- Pinelli E, Brandes S, Dormans J, Gremmer E, van Loveren H (2008) Infection with the roundworm *Toxocara canis* leads to exacerbation of experimental allergic airway inflammation. Clin Exp Allergy 38: 649–658
- Pinelli E, Herremans T, Harms MG, Hoek D, Kortbeek LM (2011) *Toxocara* and *Ascaris* seropositivity among patients suspected of visceral and ocular larva migrans in the Netherlands: trends from 1998 to 2009. Eur J Clin Microbiol Infect Dis 30:873–879
- Pullola T, Vierimaa J, Saari S, Virtala AM, Nikander S, Sukura A (2006) Canine intestinal helminths in Finland: prevalence, risk factors and endoparasite control practices. Vet Parasitol 140:321–326
- Sager H, Moret C, Grimm F, Deplazes P, Doherr MG, Gottstein B (2006) Coprological study on intestinal helminths in Swiss dogs: temporal aspects of anthelminthic treatment. Parasitol Res 98:333–338
- Strube C, Heuer L, Janecek E (2013) *Toxocara* spp. infections in paratenic hosts. Vet Parasitol 193:375–389
- Warren EG (1969) Infections of *Toxocara canis* in dogs fed infected mouse tissues. Parasitology 59:837–841
- Williams RL (2000) A note on robust variance estimation for clustercorrelated data. Biometrics 56:645–646