Intensity of *Toxocara canis* shedding of foxes in the North-Eastern part of the Netherlands

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Abstract

Objectives

To determine the prevalence and intensity of *Toxocara canis* infection in foxes in the Netherlands and to investigate associations between *T. canis* presence, and eggs per gram (EPG), EPG per female worm (EPG/FW) and age, gender and body-weight-length (BWL) index.

Methods

Between October 2016 and January 2017, 69 foxes were shot in the north-eastern part of the Netherlands. Before dissection on these foxes, data on age, gender, weight and length was collected. During dissection, faeces and *T. canis* worms were collected. The worms were counted and sexed using a stereomicroscope. The Centrifugal Sedimentation Flotation (CSF) method was used for faecal examination, and the McMaster technique to determine the EPG when *T. canis* eggs were present.

Results

The prevalence of *T. canis* was 63,8%, the mean EPG was 472, and the mean EPG/FW was 78. No significant relations were found between *T. canis* presence, EPG, EPG/FW, and age, gender or BWL-index. One *T. canis* worm was found inside the right atrium of a fox's heart.

Clinical significance

The EPG/FW of *T. canis* in Dutch foxes has been determined for the first time and is a prelude to a more quantified collection of data regarding *T. canis* infections in animals. This study provides a first indication about the intensity of infection and the actual contribution of foxes to environmental contamination with *T. canis* eggs in the Netherlands.

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Introduction

Toxocara canis is mostly known as a zoonotic roundworm of dogs. In humans, infection with *T. canis* can cause a variety of clinical syndromes, described as Covert Toxocarosis (CT), Visceral Larva Migrans (VLM), Ocular Larva Migrans (OLM) (1), and Neurological Toxocarosis (NT) (2,3). People generally get infected by ingestion of embyronated eggs from a contaminated environment (4). Since dogs are the largest contributors to environmental contamination with *T. canis* eggs (5), most studies focus on *T. canis* in dogs and prevention of environmental contamination with *Toxocara* eggs.

However, foxes, being also a source for environmental contamination with *T. canis* eggs, are often neglected. Only a few studies about *T. canis* in foxes are reported, and most of them focussed on prevalences and mean intensity. As presented in Table 1, the prevalence of *T. canis* in foxes varies greatly between European countries.

Table 1 Overview of prevalences of T. canis in foxes in various European countries

Prevalence (%)	Country	Study
9,1	Italy	Magi et al. 2009
19 – 37,7	Ireland	Stuart et al. 2013; Wolfe et al. 2001
25,5	Belarus	Shimalov 2003
28,23	Croatia	Rajkovic-Janje et al. 2002
31,3	Germany	Loos Frank, Zeyhle 1982
38,3	Slovenia	Vergles Rataj et al. 2013
44,3	Switzerland	Reperant et al. 2007
59,4 – 60,9	Denmark	Saeed, Kapel 2006; Al-Sabi et al. 2013
61,6	Great Britain	Smith et al. 2003
61 – 73,7	The Netherlands	Franssen et al. 2014; Borgsteede 1984

In the Netherlands, the first internationally published study on the incidence of parasites in foxes has been carried out by Borgsteede between 1978 and 1979. In this study, 139 foxes that were shot near the Dutch-German border were examined, and 137 of these foxes were suitable for parasitological investigation. Of these 137 foxes, 73,7% was found to be positive for *T. canis* (18). For over 30 years this was the sole report on helminth species in Dutch foxes, until Franssen et al. examined 136 foxes that were also shot along the Dutch-German border in 2014. They found the number of helminth species in Dutch foxes to be increased overall. When comparing their data on the prevalence of *T. canis* in foxes (61%) with the prevalence found by Borgsteede (1984), the prevalence showed a decrease with 17% over a 35-year period (17).

Since these two studies are the only ones to have studied infections with helminths in foxes in the Netherlands, and both studies only determined prevalence, a lot of important data on helminths and with special interest *T. canis* in Dutch foxes is lacking. Prevalence alone is not sufficient to determine the contribution of foxes to the contamination of the environment with *T. canis* eggs and the risk for both dog and human infection. Therefore, a more quantitative approach is needed.

Morgan et al. (2013) tried quantifying environmental contamination with *Toxocara* eggs by using estimates of the host population density and infection levels in Bristol, UK (19). Building on this study, Nijsse et al. (2015) developed a model to estimate the relative contribution of, inter alia, foxes to the contamination of the environment with *Toxocara* eggs in the Netherlands. Based on assumptions in the models, they found that foxes overall contribute relatively little (14,9%) to environmental contamination with *Toxocara* eggs, compared to the main shedders; dogs (39,1%) (5). In rural areas, however, foxes, with 41,3% infection rates, did account most to the in total 1.05 x 10^9 eggs shed per

day in those areas (5). In this model, an average of 157 eggs per gram (EPG) for foxes six to twelve months old, and an average of 366 EPG for foxes over twelve months old were used (5). These means were taken from a study by Saeed & Kapel, who examined *T. canis* in Danish red foxes between 1997 and 2002 (14), since such data was not available at the time for red foxes in the Netherlands. Even though Nijsse et al. (2015) provided an insight into the relative contribution of foxes to environmental contamination with *T. canis* eggs, the intensity of infection and therefore the actual contribution of Dutch foxes remains unknown.

The aim of this study was to determine the intensity of *T. canis* infections in foxes in the Netherlands, expressed in eggs per gram (EPG) and per female worm (EPG/FW), present in the small intestines and cecum. This could be used for further research for quantifying environmental contamination of foxes with *T. canis* eggs. In addition, relations between *T. canis* presence, EPG and EPG/FW with age, gender and body-weight-length (BWL) index were assessed, which could be used for further research into the demographic backgrounds of *T. canis* infection in Dutch foxes.

Materials and Methods

Over the period October 2016 to January 2017, hunters regularly shot foxes in the provinces of Groningen and Drenthe. The hunters were asked to send 120 of these foxes to the National Institute for Public Health and the Environment (RIVM, Bilthoven, The Netherlands), where the foxes were stored for at least a week at -80°C to inactivate any eggs present of *Echinococcus multilocularis* (20).

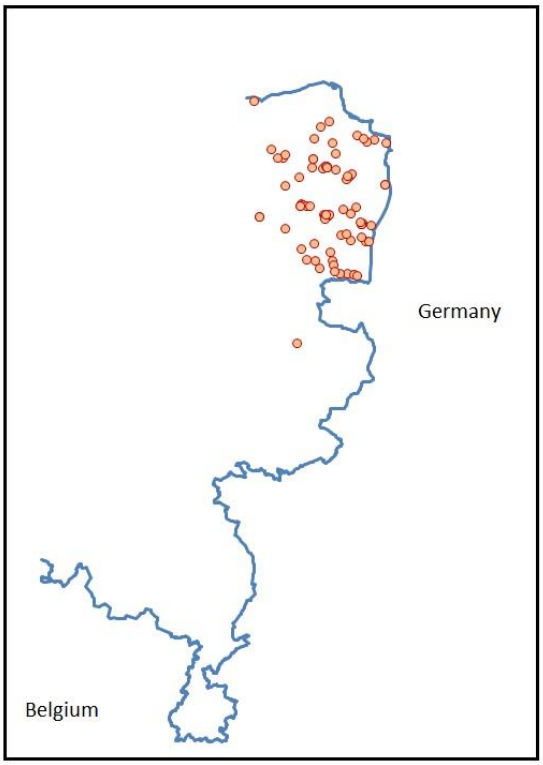

Figure 1 Map showing the eastern border of The Netherlands. Geographic locations of shot foxes used in this study are indicated by an orange circle (Image: Frits Franssen with compliments)

The foxes were thawed before dissection, and data on age, gender, length (measured from nose to anus) and weight was collected. Based on an age identification chart (figure 8 appendix) from the Dutch Wildlife Health Centre (DWHC, Utrecht, The Netherlands), tooth wear of the lower incisors and either

the presence or absence of a bulb on the base of the canines were used to determine whether each fox was younger or older than one year.

During dissection, faeces, when present, was collected from the distal colon and rectum. Furthermore, the intestinal tract from pylorus to proximal colon was removed and sent to the RIVM for mucosal scrapings to collect adult *Toxocara* worms. If the intestinal tract had been perforated by buckshot pellets, any worms found freely in the abdomen were also collected.

All *T. canis* worms found by the RIVM during mucosal scrapings of the intestinal tract were collected, counted and investigated to determine their gender using a stereomicroscope. Male *T. canis* worms were identified by their curling tail and smaller overall size (21), and by having spicula (22) and a digitiform appendix on the tail-end (22,23). Female *T. canis* worms were identified based on having uteri containing eggs, a smooth narrowing tail-end (22) and by their larger overall size (21).

To determine the eggs and oocysts present in the faecal content of each fox, the Centrifugal Sedimentation/Flotation (CSF) technique was used. Three grams of faeces were suspended in tap water. After the suspension was riddled, a centrifuge tube was filled with about 10ml of the product. After centrifuging for two minutes with 1358 g Max RCF, the supernatant was drained and a sucrose solution with a density of 1,28-1,3 g/cm^3 was added as a flotation medium. After the centrifuge tube was filled to a convex meniscus with the sucrose solution, a cover slip was placed on top of the tube and the product was centrifuged for another two minutes with 1358 g Max RCF. The slides were then examined microscopically.

When the CSF was positive for *T. canis*, the McMaster technique was used to quantify the number of eggs of *T. canis* per gram faeces. Three grams of faeces were suspended in 42ml sodium chloride (NaCl) and riddled. When less than three grams of faeces was available, the amount of NaCl millilitres was adjusted accordingly to provide the same faeces-NaCl ratio. The suspension was then swirled just before filling two chambers of the McMaster. *T. canis* eggs were counted microscopically and multiplied by 50 to calculate the number of eggs per gram faeces.

Results

In total, 69 foxes were examined and 44 of them (63,8%, 95%CI 52,3-75,2) were positive for *T. canis* (Table 2). The mean EPG was 472 (95%CI 286-657) and the mean EPG/FW was 78 (95%CI 50-106) (Table 3). The faeces of one fox contained a non-typical cestode egg with a pyriform apparatus, and in the faeces of another fox a non-typical ascarid egg, possibly *Parascaris* sp., was found. Moreover, amongst the trematode eggs, eggs of *Alaria* sp. were present, but this was not further determined to species level. Furthermore, larvae of *A. vasorum* and *C. vulpis* were seen in the faeces of some foxes, along with larvae that could not be ruled out as being parasitic but were affected by the freeze-thawing process and could not be morphologically determined up to the genus level (Table 2).

Table 2 Overview of parasitic helminth eggs, larvae and coccidia found in faeces of Dutch foxes

		Number positive (n = 69)	Prevalence (%)
Nematodes:			
	Toxocara canis	44	63,8
	Capillaria spp.	43	62,3
	Strongyle type egg	40	60
	Trichuris sp.	4	5,8

	Strongyloides sp.	4	5,8
	Non-typical ascarid egg	1	1,4
Cestodes:			
	Taenia sp.	4	5,8
	Non-typical cestode egg	1	1,4
Trematodes:			
	Trematode spp.	20	29
Coccidia:			
	Eimeria spp.	2	2,9
	Eimeria sp. / *Isospora* sp.	13	18,8
	Hammondia sp. / *Neospora* sp.	1	1,4
Other:			
	Angiostrongylus vasorum larvae	2	2,9
	Crenosoma vulpis larvae	4	5,8
	Undetermined larvae	30	43,5

Table 3 Prevalence (%), egg excretion and number of female T. canis in Dutch foxes

Foxes	*# examined*	*Prevalence (%)*	*Mean EPG*	*Total female worms*	*Mean female worms*	*Mean EPG/FW*
Age < 1 year	48	68,8	532	128	5	86
Age > 1 year	21	52,4	291	13	2	51
Male	40	72,5	571	121	5	82
Female	29	51,7	280	20	3	68
BWL 5.0 – 6.9	26	61,5	344	43	3	83
BWL 7.0 – 8.9	32	62,5	583	63	5	81
BWL 9.0 – 10.9	10	70,0	186	10	3	52
Total	69	63,8	472	141	4	78

Age

Most of the foxes (69,6%) were younger than one year old. No significant difference was found between the age-category of the foxes and the presence of *T. canis* (χ^2, $P > 0.05$), the EPG (Figure 2) (T test, $P > 0.05$) and EPG/FW (Figure 3) (T test, $P > 0.05$).

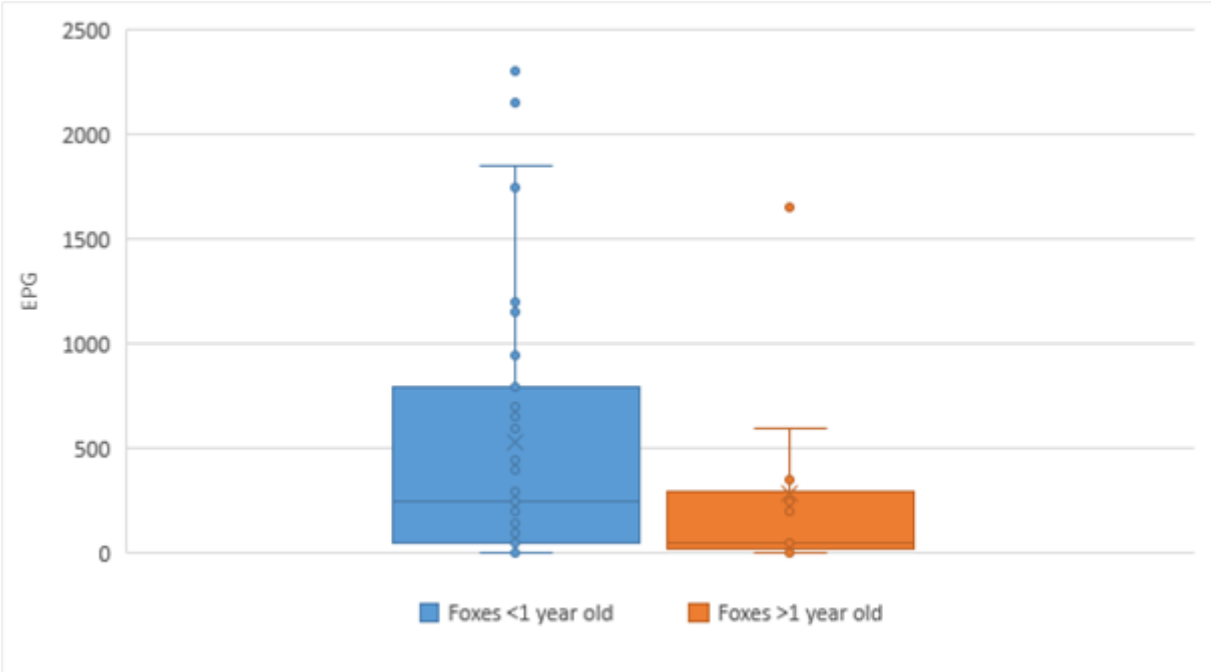

Figure 2 Box-and-whisker plot of EPG in foxes of two different age categories

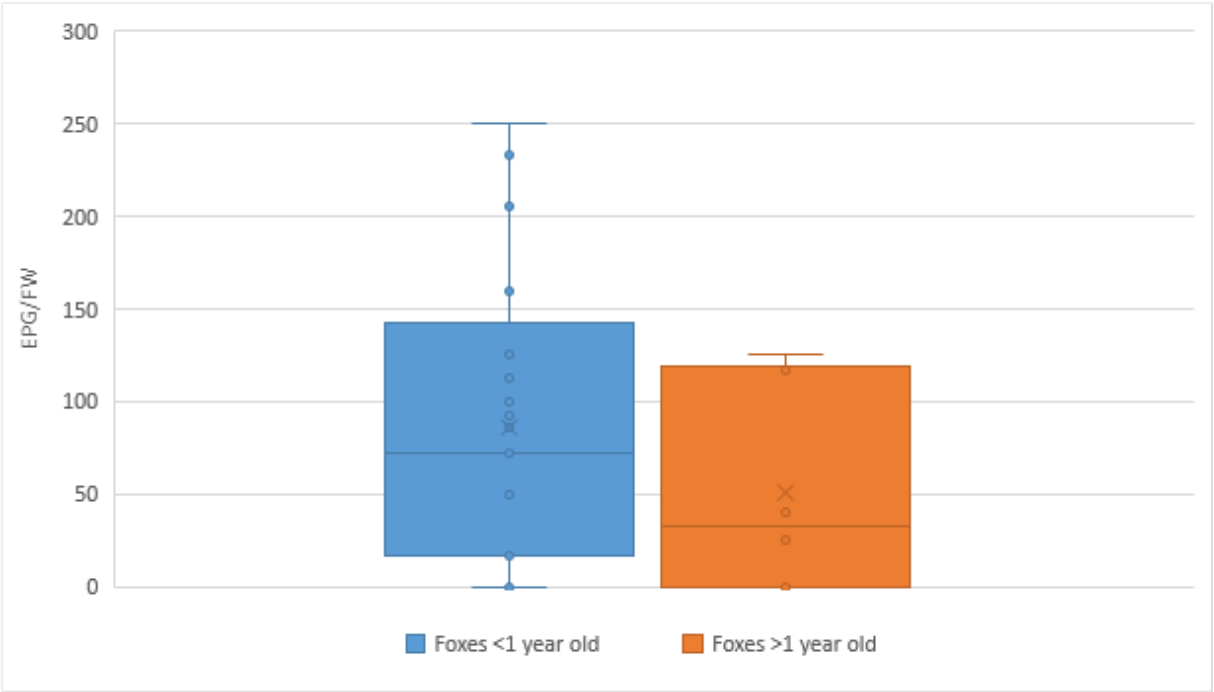

Figure 3 Box-and-whisker plot of EPG/FW in foxes of two different age categories

Gender

No significant difference was found between gender of the fox and the presence of *T. canis* (χ^2, $P > 0.05$). There was no homogenous variance between gender and EPG (F test, $P = 0.026$), but no further significant difference between gender and EPG was found (Figure 4) (Wilcoxon Rank Sum test, $P > 0.05$). Furthermore, no significant difference was found between gender and EPG/FW (Figure 5) (T test, $P > 0.05$).

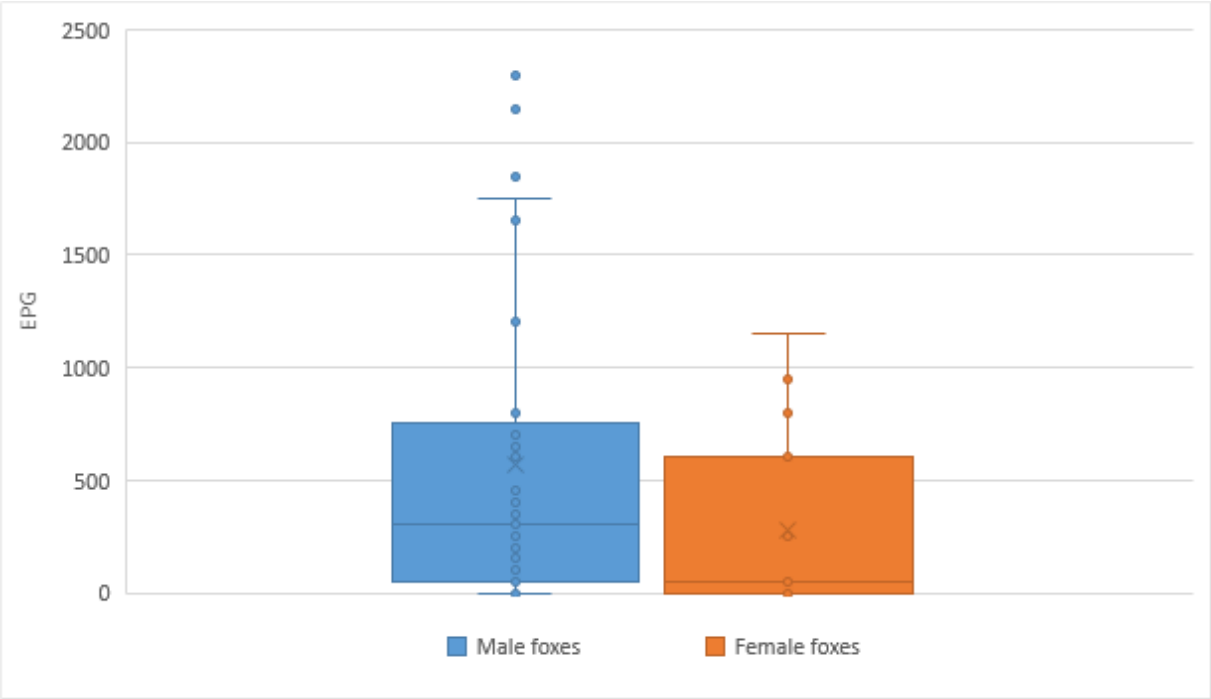

Figure 4 Box-and-whisker plot of EPG in male and female foxes

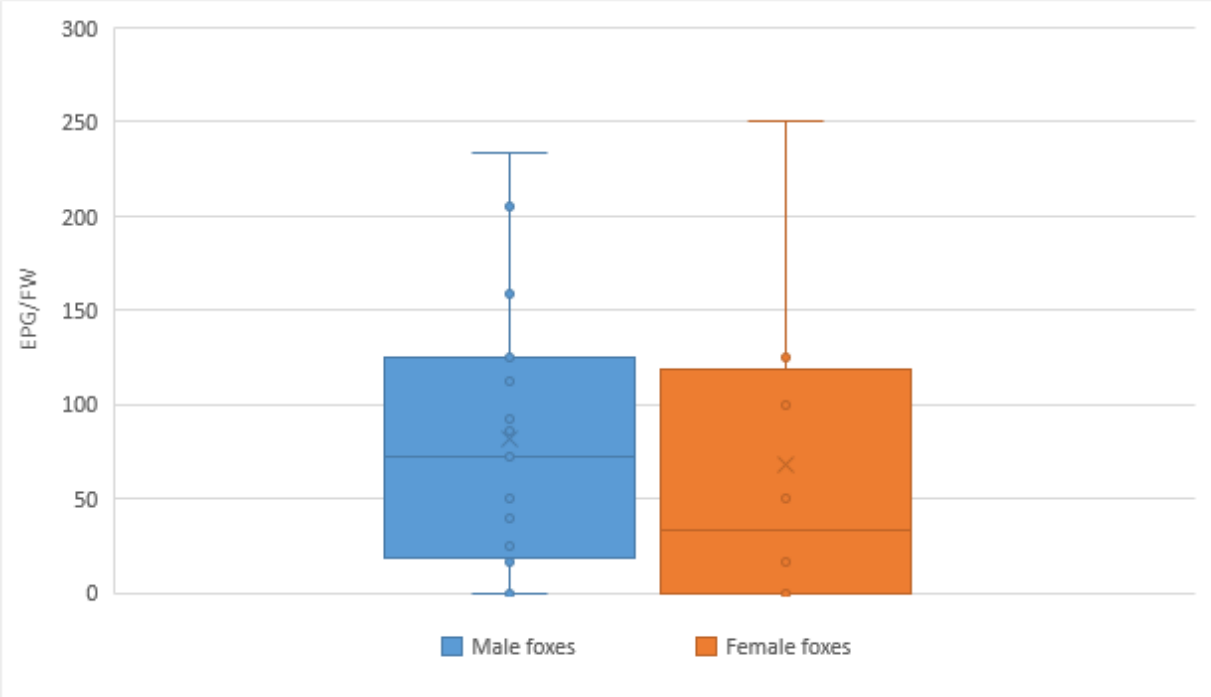

Figure 5 Box-and-whisker plot of EPG/FW in male and female foxes

BWL-index

No significant difference was found between the three BWL-index groups and the presence of *T. canis* (χ^2, df = 2, P > 0.05). Furthermore, no significant difference was found between BWL-index and EPG (figure 6) (One-way ANOVA, P > 0.05), and between BWL-index and EPG/FW (figure 7) (One-way ANOVA, P > 0.05).

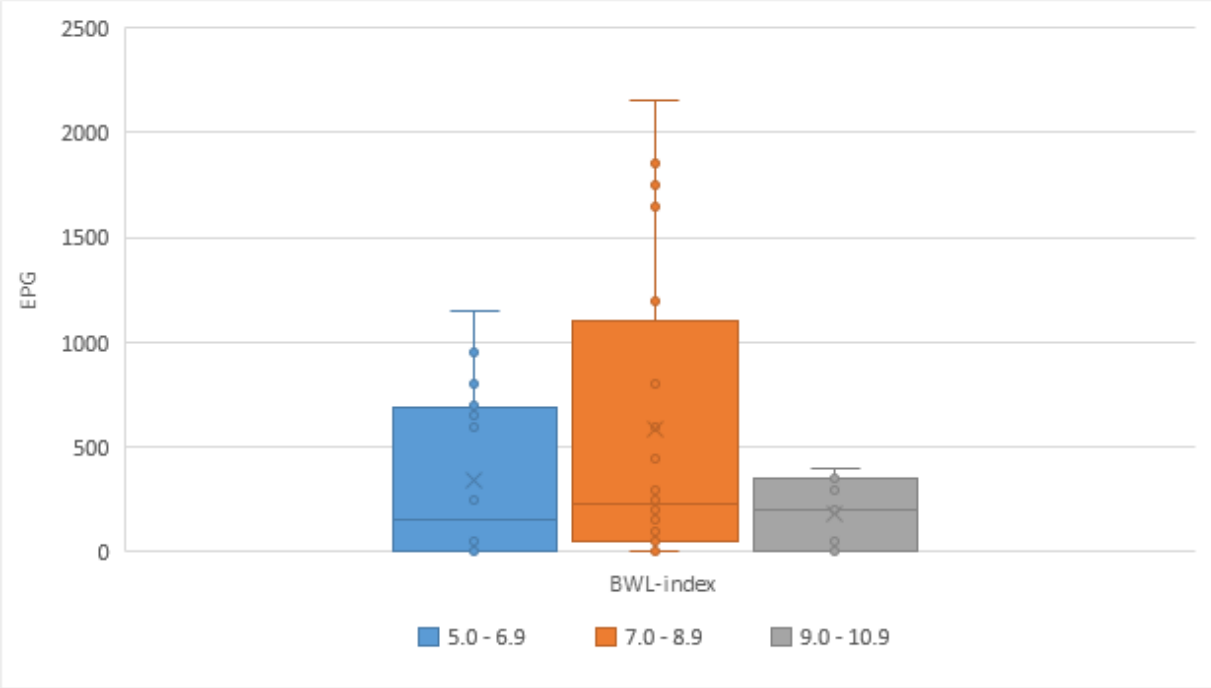

Figure 6 Box-and-whisker plot of EPG in foxes of three different BWL-index groups

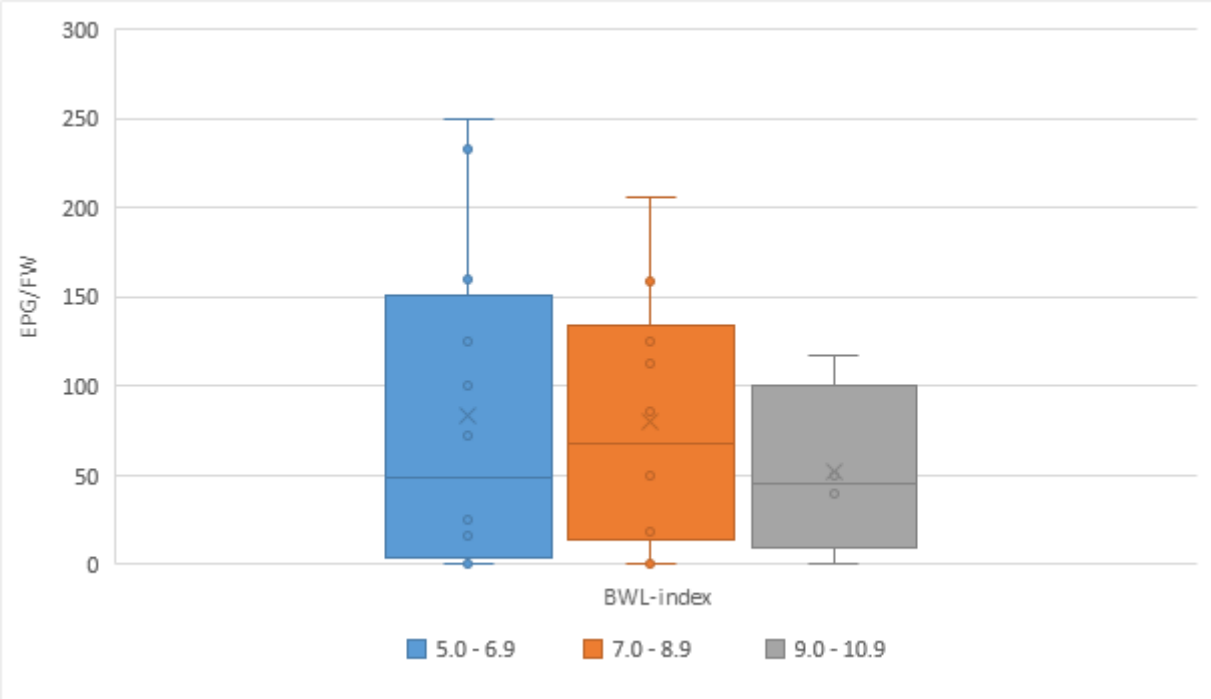

Figure 7 Box-and-whisker plot of EPG/FW in foxes of three different BWL-index groups

Additional finding

In one case, during dissection, two *T. canis* worms were found in the thoracic cavity, but more surprisingly, one *T. canis* worm was found inside the right atrium of the heart. A similar case has only once been reported before, in which a male *T. canis* worm was found in the coronary groove at the junction of the right atrium and ventricle of a fox's heart (24).

Discussion and Conclusion

In this study, the prevalence of *T. canis* in Dutch foxes was found to be 63,8%, with a 95% confidence interval between 52,3% to 75,2%. This is in line with previously found prevalences in The Netherlands of 73,7% and 61% by Borgsteede (1984) and Franssen et al. (2014) respectively.

The average egg output of *T. canis* was determined to be 472 EPG, which seems to be slightly higher than the mean EPG's of respectively 411 and 394 reported by Saeed and Kapel (2006) and Sowemimo (2007). Although the calculated 95% confidence interval of 286 to 657 amply covers these EPG's, some small differences could be due to this study using a McMaster method with a detection limit of 50 EPG, whereas Saeed and Kapel (2006) used a McMaster method with a more precise detection limit of 20 EPG (14).

The mean EPG/FW was calculated to be 78. When combining this data with the mean number of female *T. canis* worms per fox and the mean faecal excretion per fox per day, it is possible to estimate the average daily contribution of foxes to environmental contamination with *T. canis* eggs. Since this study found an average of 4 female worms per fox (Table 3), and Nissen et al. (2013) found a mean faecal excretion of 95g per fox per day (26), this would come down to an average of 29.640 *T. canis* eggs shed per fox per day. However, it should be noted that in this study the number of female worms per fox could be higher, since there is no certainty all worms were collected during dissection, whereas the number of EPG could be lower due to the detection limit of the McMaster being high, as mentioned earlier. Moreover, for ease, all *T. canis* worms in this study were considered to be sexually mature. Furthermore, Nissen et al. (2013) used only 5-month-old female foxes to calculate the average faecal output per day (26), which is not a valid representation of a random, diverse fox population.

Franssen et al. (2014) reported that 70% of the shot foxes were 7 to 12 months old (17). In this study, it was confirmed that most of the foxes (69,6%) were younger than one year old (Table 3). However, the age of the foxes in this study was only determined by examining tooth wear, whereas Franssen et al. (2014) also microscopically examined slices of teeth for a more precise age estimation. Although tooth wear alone does not give a very accurate estimation of the fox's age, since it is also dependent on the diet of the fox (27), it was the preferred method in this study because it is less time consuming than microscopic examination.

No significant relations were found between *T. canis* presence, EPG, EPG/FW, and age, BWL-index, or gender. Franssen et al. (2014) did neither find a correlation between BWL-index and infection classes, but determined a significant higher prevalence of *T. canis* in male foxes compared to female foxes (17). Because this study examined just a relatively small number of foxes (n = 69) compared to the 262 foxes examined by Franssen et al (2014), it is possible there was insufficient data to properly demonstrate any relations.

During dissection, a *T. canis* worm was discovered inside the right atrium of a fox's heart. Although this is a remarkable finding, intravital presence of this worm in the heart could not be verified. In the same fox, two *T. canis* worms were found freely in the thoracic cavity while the thoracic diaphragm seemed to be intact. This suggests that either the oesophagus was shot open and *T. canis* worms were regurgitated by the dying fox or moved post mortem (whether by human handling of the fox's body) outside of the oesophagus, or either contamination occurred during dissection. The same explanations apply for the *T. canis* worm found in the heart, although it seems unlikely the worm would have made such a sophisticated journey from the digestive tract to the heart post mortem.

In conclusion, the prevalence of *T. canis* does not differ from previous studies conducted in foxes in the Netherlands. This study shows that the EPG seems to be slightly higher than reported in previous studies. Furthermore, in the present study, the EPG/FW for *T. canis* in foxes has been calculated for the first time and was determined to be 78, which is a prelude to more quantified collection of data regarding *Toxocara* infections in animals. As shown before, the EPG/FW can be used to calculate the average eggs shed per female *T. canis* worm per animal per day, giving a good indication of the contribution of an animal to environmental contamination with *T. canis* eggs. This study can be built upon by examining a greater number of foxes and studying the weight of fox faeces from a larger, more diverse population. Also, by expanding this study to include dogs, and to include foxes from multiple parts of the Netherlands with different grades of urbanisation, a more complete view on environmental contamination with *T. canis* eggs in the Netherlands can be provided.

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Appendix

Figure 8 Age identification chart used for determining the age of foxes. DWHC. Available on: https://www.dwhc.nl/wp-content/uploads/2016/02/VOSSEN_leeftijdbepaling_2016.pdf

Figure 9a Curled tail-end of male T. canis

Figure 9b Tail-end of female T. canis