A critical reflection on current control of *Toxocara canis* in household dogs

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Printing of this thesis was financially supported by:
Faculty of Veterinary Medicine, Utrecht University
Infection and Immunity Center Utrecht
AUV

Illustrations in this thesis are reprints of etchings by Wim Hendrikx 2016
met dank aan de Afdeling Multimedia van de Faculteit Diergeneeskunde
Cover: *Toxocara* eggs , line etching/aquatint
Chapters: Developing *Toxocara* eggs, line etching/aquatint

Printed by: Atalanta Drukwerkbemiddeling, Houten
ISBN/EAN: 978-90-393-6675-2

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Een kritische beschouwing op de huidige adviezen ter bestrijding van *Toxocara canis* in huishonden

(met een samenvatting in het Nederlands)

proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht,
op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op
donderdag 24 november 2016 des middags te 12.45 uur

door

Eize Roelof Nijsse
geboren op 14 december 1969 te Middelburg

Promotor:	Prof. dr. J.A. Wagenaar
Copromotoren:	Dr. ir. H.W. Ploeger Dr. L. Mughini Gras

Voor mijn ouders

INHOUDSOPGAVE

Chapter 1
General Introduction

Illustratie: Wim Hendrikx
Toxocara I
lijnets/aquatint 2016

In many industrialized countries, pets and particularly dogs are more and more considered as full-fledged family members. This leads to a living environment that is closely shared between dogs and their owners. Both the dog and the environment can be sources of zoonotic infections. There are many infectious diseases in companion animals for which effective control programs exist. For other diseases these programs are still lacking even though such infections may affect the health of the animal itself, as well as pose a threat to public health. Infections with the roundworm *Toxocara* sp. in dogs and cats are an example of such infections whose current control programs have a limited scientific basis.
Toxocara larvae that migrate through the human body may lead to ocular larva migrans (OLM), visceral larva migrans (VLM), including health problems like exacerbation of asthmatic complaints and possible neurologic effects, and covert toxocariasis (CT) (Beaver et al. 1952; Dent et al. 1956; Pinelli et al. 2008; Pinelli and Aranzamendi 2012).

During the last decades, the prevalence of dogs shedding *Toxocara canis* eggs did not decrease significantly in the Netherlands and neighboring countries (Overgaauw 1997a; Claerebout et al. 2009; Overgaauw et al. 2009; Barutzki and Schaper 2011). This suggests that the advocated *T. canis* control programs in dogs, which mainly aim at the prevention of human infections, appear to be less effective than anticipated. Most existing control programs focus on regular blind deworming of household dogs. This is based on the notion that patent *T. canis* infections occur occasionally in adult dogs (Sprent 1958; Visco et al. 1977; Lloyd 1993; Sager et al. 2006; Overgaauw and Van Knapen 2013). However, the biology of patent infections with *T. canis* is still largely unknown, and so many questions about the epidemiology of the disease in dogs and humans are still unanswered. Additionally, many questions remain concerning the interaction between canid host and parasite. Therefore, the reasons as to why this parasitic infection appears to be so hard to control might be found in the biology of *T. canis* itself. Critical points are, for instance, the longevity of eggs in the environment and the versatility of the larval stages in both final and paratenic hosts.

BOX 1

The terminology used for infections with, and disease due to, *Toxocara* sp. lacks uniformity, which can lead to confusion in defining the burden of illness and aims for controlling both infection and disease.

In 1959, Whitlock proposed to use the suffix "–osis" for disease caused by a parasitic infection and "–iasis" for indicating the asymptomatic, relatively lesion-less, carrier state (Whitlock 1959). The use of this terminology makes sense for *Toxocara* infections, both in dogs and in humans, because infection with this parasite does not necessarily mean that symptoms are present. SNOPAD (Standardized NOmenclature for PArasitic Diseases) advocates the use of uniform disease names (-osis) for parasitic infections, which does not discriminate between mere infection and disease (Kassai 2006).

However, in most of the literature dealing with *Toxocara* infections in humans, toxocariasis is used to indicate *Toxocara* infections both with or without symptoms in humans and toxocarosis for infections with or without disease in animals. This thesis aims to inform both veterinary and medical professionals, therefore –iasis is used in the general introduction and discussion for infection in humans and –osis for infection in animals. In the different chapters, the terminology is used according to the guidelines of the journals the papers were submitted to. The author however recognizes the need for a uniform use of the terminology, preferring one that can distinguish between mere infection and disease.

Biology of *Toxocara canis* (Werner, 1782)

The adult stages of *T. canis* reside in the intestines of the dog and other canids. When these adult stages become reproductive, a patent infection starts and large numbers of characteristically thick-walled eggs (Fig. 1) are produced and shed into the environment with the dog's faeces. The eggs need to develop in the environment to the "infective stage", in which the larva has moulted twice to become a third-stage larva (L3). If a dog that has not yet developed an effective immune response to *T. canis* ingests these embryonated eggs, they will hatch in the small intestine. The emerging larvae penetrate through the intestinal mucosa to be transported with the bloodstream to the liver, heart and finally to the lungs where they can actively invade the lung tissue and migrate to the upper respiratory tract. Here, the larvae are coughed up, swallowed, and conveyed to the intestines again where they undergo their last moult to L5, the young adult stage, before maturing to the adult reproductive stage. This hepato-tracheal migration route occurs in dogs in the absence of a functional immunological response to the migrating larvae, and this is the dominant route in dogs younger than 3 months of age. These young dogs are considered to shed most of the *Toxocara* eggs (Greve 1971; Morgan et al. 2013). This is not only due to the

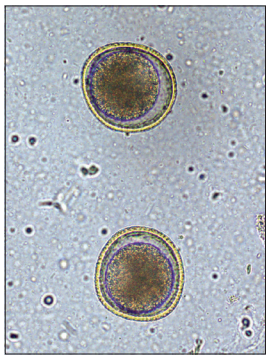

Fig. 1. Toxocara canis eggs.

lack of a functional immunological response against migrating larvae in their body after ingesting infective eggs, but also -and perhaps even more importantly- due to two other routes of infection. First, puppies already become infected during pregnancy. Reactivated larvae in a pregnant bitch migrate to the uterus and cross the placenta to enter the umbilical bloodstream. In the unborn fetus, the larvae migrate towards the liver and the lungs. This leads to a patent infection after birth when the pup is about 16 days old (Lloyd 1993). Second, reactivated larvae in the dam also migrate to the mammary glands and larvae are passed with the milk to the suckling puppies, the so-called lactogenic route of infection (Burke and Roberson 1985). After ingestion with the milk, the larvae are thought to mature directly in the intestine without further migration in the body. However, this last part of the process is questioned because of the rather long prepatent period (27-35 days) after lactogenic infection (Schnieder et al. 2011). For cats, however, a shortened prepatent period after lactogenic infection with larvae of *Toxocara cati* has been reported (Sprent 1956). Partly due to these vertical routes of infection, it is likely that puppies indeed contribute the most to the environmental contamination with *Toxocara* eggs (Morgan et al. 2013). When constrained to the litter area, the nursing bitch can spread the yet non-infective eggs via her faeces as passers after cleaning up the puppies' faeces. In addition, as the faeces of the puppies also may contain viable larvae, this could also lead to a patent infection in the nursing bitch herself (Sprent 1961).

If a dog with an effective immunological response to *T. canis* gets infected with embryonated eggs, the same process of hatching in the intestine and entering the bloodstream takes place. However, the larvae will not be able to pass through the lung tissue and will be transported back with the bloodstream to different somatic tissues. Here they will become dormant for many years. Larvae have been recovered from muscles, kidney, liver, lungs, thyroid, pituitary gland, retina, popliteal lymph node, mesenteric lymph node, pancreas, myocardium, intestine, brain and cauda equina (Barron and Saunders 1966). The effect of this immunological response is called "age resistance", which is likely the result of both a matured immune competence as such and acquired immunity due to the infection in the first months of life (Barriga 1988). This starts to develop when puppies are about three months of age and most of the dogs are considered to have developed resistance at the age of six months. In these older dogs, infection with infective eggs is less likely to lead to patent infections, or at least not directly following the prepatent period known in puppies. Therefore, when their faeces is not properly disposed of, a dog older than

six months is less likely to contribute significantly to the environmental contamination with *Toxocara* eggs compared to a younger dog. They will, however, harbour somatic larvae. Although these larvae will rarely cause disease, they might occasionally cause granulomatous inflammations to the tissue (Barron and Saunders 1966). A similar route of infection is also seen when non-canids, including humans, get infected with *T. canis*. In these hosts, larvae cannot pass the lungs and therefore are not able to finish the hepato-tracheal migration and end up as dormant larvae in somatic tissues. Such accidental hosts are called paratenic hosts, which can play an important role in the lifecycle of *T. canis*.

When a dog older than 6 months (referred to as "older dogs" later in the text) starts shedding *Toxocara* eggs, this can be explained in two ways. First, dormant larvae can be reactivated under the influence of some circumstances compromising the immune system (Lloyd et al. 1981). These reactivated larvae can continue their hepato-tracheal migration route and end up as adults in the intestines. Second, a dog may ingest a prey animal (a paratenic host) or raw or undercooked meat that contains viable larvae that can develop to the adult stage with or without migrating out of the intestines and therefore do not necessarily need to pass through the lungs (Sprent 1958; Warren 1969; Overgaauw 1997a). Consequently, they can avoid any immune reaction in the lungs and can become reproductive in the intestines. Overall, for household dogs in the Netherlands, of which the majority is older than 6 months, the reported prevalence of patent infections is about 5% (Overgaauw 1997b; Overgaauw et al. 2009).

Toxocara infections in humans

As mentioned above, *Toxocara* can infect many species of paratenic hosts including humans, which is the main reason for the worldwide focus on controlling *Toxocara* infections in dogs. Infection will not lead to adult roundworms living in the intestines of a human being, but to third stage larvae residing in somatic tissues. These somatic larvae are assumed to survive in a dormant state for up to 6-10 years (Beaver 1962; Strube et al. 2013), most of the time without causing noticeable symptoms (Fillaux and Magnaval 2013). If the presence of these larvae results in symptoms in humans, this is called toxocariasis (see Box 1). Toxocariasis is considered a neglected disease and in some countries a poverty related disease (Won et al. 2008; McGuinness and Leder 2014). In industrialized countries, low socio-economic status does not always seem to be related to, but sometimes even appeared to be protective for infection with *Toxocara*, but this can vary with degrees of urbanization (Mughini-Gras et al. 2016). Higher seroprevalences are sometimes observed in rural areas (Uhlikova and Hubner 1998; Deutz et al. 2005; Strube et al. 2013).

Ingestion of contaminated soil is thought to be the most important route of infection in humans. This implies that consumption of raw vegetables can pose a risk (El Said Said 2012; Rostami et al. 2016). Infection through direct contact with a dog's fur because of the adhesive character of *Toxocara* eggs has been discussed in several publications (Roddie et al. 2008; Keegan and Holland 2010; Nagy et al. 2011). It was concluded by Overgaauw et al. (2009) that no, or only few, embryonated eggs can be detected in the fur of household dogs, which was confirmed in a more recent study (Paoletti et al. 2015). This makes it very unlikely that this is an epidemiologically relevant source of infection. Similarly, eggs may be transported through hands, clothes, picnic blankets, and other mechanical means. Little is known about the importance of such transmission routes. There are also some reports of infection after eating raw or undercooked meat or organs of paratenic hosts (lamb, chicken, beef) that contained somatic larvae (Nagakura et al. 1989; Salem and Schantz 1992; Taira et al. 2004; Yoshikawa et al. 2008; Yang et al. 2014), but the actual relation between infection and consumption of raw meat in these cases is more assumptive than proven. Although this route of infection has been reported in mice and pigs (Tüzer et al. 2002; Taira et al. 2004), it is not clear if eating meat from paratenic hosts can lead to migrating larvae in humans. An unusual case of toxocariasis reported in the literature has been attributed to the ingestion of raw slugs as an alternative therapy for gastric ulcers (Fellrath and Magnaval 2014).

To the knowledge of the author, there are no publications about the relation between infection dose and severity of disease in humans. There have been studies in mice (Holland and Cox 2001), but results are difficult to extrapolate to humans. In theory, one migrating larva can cause harm if it ends up in the "wrong place", like the brain or the eye, or if it triggers a hypersensitivity response in, for example, the lungs (Buijs et al. 1997; Pinelli et al. 2008; Pinelli and Aranzamendi 2012). Disease attributed to migrating larvae is mostly found in children because of their higher probability of exhibiting geophagia (eating soil/sand), but it can occur at any age. Exposure of humans is studied by measuring specific antibodies directed against the excretory secretory products of *Toxocara* larvae (Magnaval and Glickman 2006; Smith and Noordin 2006). The prevalence of seropositive humans is about 8-10% in the Netherlands and increases with age from childhood onwards suggesting a continuous exposure (Mughini-Gras et al. 2016).

In 2001, it has been proposed to summarize the effects of migrating or dormant larvae in four clinical forms: (i) systemic forms, which include classical VLM and incomplete VLM; (ii) compartmentalized forms, which include ocular and neurological toxocariasis (OT and NT); (iii) CT; and (iv) asymptomatic toxocariasis (Pawlowski 2001). Nowadays, the compartmentalisation as such is less emphasized and clinical

forms are usually defined as OLM or OT, VLM, CT, and cerebral toxocariasis or NT. Most infections will probably pass without noteworthy symptoms and therefore undetected. Consequently, Pawlowski's asymptomatic fourth group is likely underestimated and probably the most prevalent one. Serological studies in the general population, without symptoms, support this (Hayashi et al. 2005; Walsh and Haseeb 2012; Mughini-Gras et al. 2016). Because the other forms do lead to symptoms that interfere with a patient's well-being, they will be discussed briefly as this defines the necessity for a control program. In most of the reported clinical cases, *T. canis* larvae are held responsible for the infection. However, this is based on serological tests that prove the presence of larvae of the genus *Toxocara*, but do not discriminate between *T. canis* and *T. cati*. Because, for example, larvae of *T. canis* appear to be more prone to migrate to the brain of mice than those of *T. cati* (Janecek et al. 2014), it is often assumed that the same happens in man and therefore this may lead to a premature conclusion that it must be *T. canis* that is involved in the process of neuro-toxocariasis.

Compartmentalized forms of toxocariasis

OT is a disease of the eye or optic nerve (Glickman and Schantz 1981). It has been observed in both children and adults (Biglan et al. 1979; Ahn et al. 2014). The prevalence ranges from 1.1% in patients visiting an eye clinic to 0.1% in the general population in Alabama (the United States of America) (Maetz et al. 1987). In Ireland, the reported estimated prevalence in school-going children is 0.01% (Good et al. 2004). In Japan, OT is thought to be the cause of 1.1% of uveitis cases (Goto et al. 2007). There is no data available on the prevalence of ocular toxocariasis in the Netherlands. The damage that leads to the symptoms is caused by *Toxocara* larvae migrating into and settling in the eye. Signs are usually reported to be unilateral. Eventually, the presence of a larva and the sequela of the patient's immune response may lead to impairment of eyesight and ultimately even in the loss of sight. A definitive diagnosis can be established by detecting the typical ophthalmologic signs, eosinophilia, anti-*Toxocara* IgG antibodies and possibly elevated IgE levels. More specifically, antibodies can be detected in the intra ocular fluid (Benitez del Castillo et al. 1995; De Visser et al. 2008). Often, OT does not lead to clinical symptoms and is only diagnosed during routine eye examinations (Good et al. 2004).

In the last decade, there appears to be a growing awareness that an infection with *Toxocara* may be responsible for neurological problems, possibly leading to impaired cognitive functions or epileptiform seizures (Walsh and Haseeb 2012; Fan et al. 2015). Field studies on this form of toxocariasis are difficult to interpret, especially when they focus, for example, on school performance in children. From experimental studies it has been reported that *Toxocara*-positive mice show changes in behaviour (Holland and Cox 2001) and impaired learning capacity and memory (Hamilton et

al. 2006). In general, it was thought that in particular *T. canis* was responsible for NT. However, in contrast with what is often assumed, it has been shown that *T. cati* is also able -albeit to a lesser extent- to migrate to the brain, at least in mice. Yet, there appears to be a difference between both species in localization in the brain. *T. canis* was found more often in the cerebra and *T. cati* in the cerebellum (Janecek et al. 2014). It is not clear what the consequences of these findings are for zoonotic cases of *Toxocara* infections. A variety of symptoms are attributed to NT, including motoric disorders, behavioural disorders, and mental and cognitive problems (Fillaux and Magnaval 2013; Fan et al. 2015). Only a few clear cases of NT have been reported. However, taking into account that the central nervous system is among the tissues where *T. canis* can be frequently detected in mice, the number of cases may be underestimated. Given that environmental contamination is common and seropositivity in the general population appears to be accumulating with age, undetected infections of the neural tissues may be more common in humans than previously suspected.

Systemic forms of toxocariasis

When the larvae reside in or pass through internal organs like the liver, kidneys or the lungs, this can lead to VLM. This will lead to less well-defined complaints requiring a more indirect way of diagnosis. Diagnosis may be supported by eosinophilia and increased levels of *Toxocara*-specific antibodies and IgE in serum. One form of VLM that is worthwhile mentioning is the possible aggravation of allergic airway inflammation, the Loeffler's syndrome (Pinelli et al. 2008; Pinelli and Aranzamendi 2012; Li et al. 2014). This syndrome is caused by pulmonary eosinophilia due to the presence in, and penetration of, the lungs of / by larvae and the accompanying immunological response. In some cases, this process remains asymptomatic or causes only peripheral blood eosinophilia, dyspnea, wheeze, and cough, but more severe cases can also occur (Pinelli and Aranzamendi 2012).

The least well defined form of toxocariasis is CT. This form concerns patients with an antibody titer against *Toxocara* showing, for example, mainly abdominal pain, fever, headache, sleep disorders, anorexia, abdominal pain, hepatomegaly, nausea, or vomiting. This can occur with or without eosinophilia (Taylor et al. 1988). Diagnosis is often based on circumstantial evidence, and demonstrating CT as the cause for observed complaints is usually performed by excluding other causes involving a thorough and extensive diagnostic work-up of the patient.

In general, toxocariasis is considered a "neglected disease", though it is likely an ubiquitous prevalent disease both in developed and developing countries (Hotez and Wilkins 2009; McGuinness and Leder 2014). A possible explanation for this is the difficulty in diagnosing the various clinical forms of toxocariasis, leading to an ill-defined

burden of illness. Moreover, even with a presumed diagnosis of toxocariasis, a long incubation period must usually be kept in mind, and it often remains difficult to unequivocally establish a cause-effect relationship between a diagnosed *Toxocara* infection and observed symptoms. An exception of this can be the ocular form, where the presence of compartmentalized antibodies in the aqueous or vitreous fluid strongly supports the diagnosis. This is also the case for neurotoxocariasis when eosinophils and/or antibodies are present in the cerebro-spinal fluid (Magnaval et al. 1997; Vidal et al. 2003).

It is clear that there are many gaps in our knowledge concerning the prevalence and incidence of disease in humans caused by *Toxocara* infection. So far, knowledge is limited to seroprevalence in the general population (Mughini-Gras et al. 2016) and among suspected toxocariasis patients (Pinelli et al. 2011) with a wide margin of error. Consequently, a more or less accurate estimate of the burden of illness, defined as disability adjusted life years (DALYs), has not been made due to lack of data.

Contamination of the environment

Whatever a dog's age or underlying reason for shedding *Toxocara* eggs, the consequence is the same. Large numbers of eggs are dispersed with their faeces into the environment. Once these eggs are shed, environmental temperature and humidity are of influence for the speed of egg embryonation (Azam et al. 2012). Under climatic circumstances as in the Netherlands, this process will take an average of three to five weeks. Once they have reached the infective stage, they are considered to be very resistant to environmental influences and can remain infective for years (Parsons 1987; Lloyd 1993). From reports about contamination of soil in public parks in different countries, it can be concluded that (viable) eggs can commonly be recovered from soil samples (Mizgajska-Wiktor and Uga 2006).

From the previous section it is clear that humans are exposed to both *T. canis* and *T. cati.* The exposure results from environmental contamination with *Toxocara* eggs in general, be it *T. canis* or *T. cati*. Therefore, one needs to consider the relative contribution of the different host species that may shed these eggs. From the household dogs, puppies and young dogs are, directly or indirectly via the lactating bitch, largely responsible for the contamination of the environment with *Toxocara* eggs (Overgaauw 1997a; Morgan et al. 2013). Besides dogs, other canids like foxes can be held responsible for the contamination of the environment. In the Netherlands, a high prevalence (73.7%) of patent infections is reported in foxes (Borgsteede 1984), probably due to predation. But also cats can shed large numbers of eggs of *T. cati* (Epe et al. 1993; Overgaauw 1997b; Robben et al. 2004). In cats, the reported preva-

lence is usually higher than in household dogs, with the highest prevalence reported in kittens and stray cats (Robben et al. 2004). In conclusion, for a proper assessment of the overall contamination of the environment with *Toxocara* eggs, one needs to consider both the numbers of potential host species and their age distribution in a given area, as well as the prevalence of *Toxocara* infection in these hosts. Reports about environmental contamination with *Toxocara* eggs are common (Gillespie et al. 1991; Ruiz de Ybanez et al. 2001; Carden et al. 2003; Avcioglu and Burgu 2008; Kroten et al. 2016). In the Netherlands, 11-25% of the soil samples of public parks in an urban area tested positive on *Toxocara* eggs (Jansen et al. 1993). Keeping in mind that the sensitivity of the detection methods used in these studies is relatively low (Ruiz De Ybanez et al. 2000), this would probably be an underestimation of the true level of contamination. Using molecular detection methods might be useful in this case (Macuhova et al. 2010).

Control of toxocarosis in dogs

The large number of very resistant eggs that are shed in the environment, combined with their zoonotic potential, call for a strategic control program to reduce patent *Toxocara* infections in dogs and/or to prevent the environment from becoming contaminated with *Toxocara* eggs.

To prevent disease in the most vulnerable group of dogs, which is also the group that probably sheds the largest number of eggs, the current deworming advice calls for a strict deworming regimen in dogs younger than six months. This way the number of adult worms in the intestines of a puppy will be controlled and by extension the number of eggs shed as well. The advised timing for deworming puppies aims at controlling patent infections due to intra-uterine and lactogenic infections, as well as infections after ingestion of embryonated eggs. Therefore, the advice is to deworm puppies every two weeks starting from 14 days of age until they are 8 weeks old, followed by monthly treatments until they are six months old. During the lactation period, the bitch is supposed to be dewormed simultaneously with the puppies (ESCCAP September 2010) because of the intake of faeces, containing larvae, of her offspring during litter care clean-up activities.

In older dogs, disease due to an infection with *T. canis* is not expected, though it cannot be excluded. The main reason to treat a possible *Toxocara* infection is preventing dogs from shedding eggs into the environment for public health reasons, as indeed patent infections are found in a proportion of adult dogs. Before 2006, the generally recommended deworming frequency for dogs older than six months was twice a year. An updated advice from the European Scientific Counsel Companion Animal

Parasites (Werkgroep Veterinaire Parasitologie Nederland 2008) intensified the advised deworming frequency to at least four times a year. The current advice of ESCCAP is to either blindly deworm a dog at least four times a year, to deworm blindly with the aid of a risk-based decision tree, or to treat based on coproscopical diagnosis (ESCCAP September 2010). In addition, there is a general recommendation to the owners of always cleaning up and disposing of their dogs' faeces, which is supported by the installation of disposal bins at various locations throughout municipalities in the Netherlands. However, there is no national legislation on this topic and, therefore, the compliance of such recommendations can differ greatly per location.

Recently, it was reported that the *Toxocara*-seroprevalence has declined, both in the general human population (Mughini-Gras et al. 2016), as in the population of patients suspected of toxocariasis (Pinelli et al. 2011). This decline has been attributed, at least partly, to the updated blind deworming advice from two to four times a year (Pinelli et al. 2011; Kanobana et al. 2013). However, in the last decade, the prevalence of patent infections in the dog population of the Benelux (Belgium-the Netherlands-Luxembourg) did not show a decline (Overgaauw 1997b; Claerebout et al. 2009; Overgaauw et al. 2009). The efficacy of the propagated deworming advice in dogs on the observed decline in human seroprevalence can therefore be questioned. It is likely that other factors such as different attitude towards personal hygiene, covering up sandboxes, and enforcement of cleaning up dog faeces play an important role in this decline. The lack of a significant decrease in prevalence of *Toxocara* egg shedding in adult dogs also requires reflection on the efficacy of the deworming recommendations from ESCCAP and raises some questions.
First, there appears to be no hard evidence that deworming blindly two or four times a year will result in fewer dogs shedding *Toxocara* eggs at some point in time. Moreover, the prepatent period in adult dogs is assumed to last at least 4 weeks. Deworming four times a year allows for ample time in-between to become infected and start shedding eggs again. Similarly, a blind deworming regimen of four times a year will likely not be effective in cases of immunocompromised dogs in which dormant larvae may become reactivated at any given point of time.
Second, owner compliance to deworming recommendations may not be complete (Overgaauw et al. 2009). The incentive of owners to deworm their dogs can influence the compliance. Treating a dog for public health reasons rather than for a dog's own health provides for a different intrinsic incentive to actually deworm a dog. Similarly, compliance to always cleaning up faeces of the dog is not complete, as the motivation for this differs when aiming for public health or when it is mostly related to dog faeces free streets and sidewalks. Evidence on the dog owners' incentives to clean up their dog's faeces is lacking (Atenstaedt and Jones 2011).
Third, prevalence data suggest that around 5% of all adult dogs shed *Toxocara* eggs

at any point in time. This implies that recommended blind deworming will result in treating circa 95% of all dogs without the actual presence of a patent infection. Dog owners and veterinarians, therefore, may question the necessity of treating individual animals without proper diagnosis. This may be further stimulated due to a changed canalization regulation of anthelmintics for horses and ruminants in the Netherlands, which may be used as support for a more critical attitude towards blind deworming by dog owners and veterinarians. Additionally there is a group of owners who are clearly pronouncing against “unnecessary” preventive health care of their animals because, according to them, this would only pose a chemical burden to the animal. This group appeals to other owners and this will not improve the compliance to any recommended deworming regimen, especially not to treatments without diagnoses.
Clearly, there is a need for a sensible alternative to the recommended blind deworming regimen. However, deworming solely based on coproscopical diagnosis is not a realistic alternative. Deworming blindly is much cheaper for an owner than a coproscopical examination for *Toxocara* eggs, even if it would indicate subsequent deworming would be unnecessary. Most anthelmintic drugs do not require any prescription and are sold by various retail outlets as over-the-counter-products without additional information. There also is no legislation to enforce pet owners to comply to deworming recommendations. Therefore, a more custom-made treatment advice for an individual animal or group of animals could lead to a higher compliance, especially when this is combined with providing information about the need to prevent patent infections because of the zoonotic risk involved.

A more custom-made advice calls for defining and assessing risk factors for patent infections, which can be used for profiling high-risk and low-risk animals. Because risk factors as predatory behaviour of the dog are probably known by the owner, a veterinarian can easily check whether or not a dog shows this behaviour or is fed raw meat from animals that can be paratenic hosts. However, impaired immunity is (unless it concerns pregnancy, prescribed medication or diagnosed illnesses) very difficult to be assessed by an owner. Risk-profiling may be accompanied by regular coproscopical examinations. Such examinations can lead to the identification of animals that shed *Toxocara* eggs more frequently than expected, for example due to temporary or permanent immune suppressive conditions. Focusing on deworming these animals may be more effective than blindly deworming only those animals whose owners comply with the advice. But even if it would be possible to prevent patent infections in all household dogs, it is not sure what the relative effect will be on the overall environmental contamination compared to the contribution by other definite host species (Morgan et al. 2013). By extension, it is unknown if a reduced relative contribution of household dogs would indeed result in a substantially lower

exposure of humans to *Toxocara* eggs and consequently a lower seroprevalence in the human population. This will depend on the distribution of cats and foxes in an area and their defecation behaviour and associated preferred locations (Uga et al. 1996). This knowledge is necessary for policymakers to assess the need for and feasibility of a control program for *T. canis.* This also calls for clarification of the actual burden of human illness. In the end, the question remains if a policy should aim for deworming all dogs at a prescribed frequency or whether it is possible to reach the same or even a better reduction with other means, for example using targeted treatment or stimulation / enforcement of cleaning up of dog faeces. To answer this question a clearer picture of the epidemiology of patent infections in household dogs is required along with proper assessments of the actual effect of deworming on environmental contamination. This includes studies to assess the relative contributions of different host populations and host species to the overall environmental contamination with *Toxocara* eggs.

All the above mentioned considerations concerning the efficacy of current control recommendations in reducing the environmental contamination with *Toxocara* eggs by household dogs, led to the work described in this thesis.

Aim of this thesis

By means of epidemiological studies, this thesis provides a critical reflection on current *T. canis* control in non-juvenile household dogs. Several questions about the epidemiology and relative importance of patent *T. canis* infections in household dogs are addressed. First, studies were performed to determine the prevalence of *Toxocara* egg shedding in dogs (**Chapter 2**), foxes (**Chapter 3**) and cats (**Chapter 4**). These studies were required to evaluate the level of infection to estimate the contribution of (household) dogs relative to that of foxes and cats to the overall contamination of the environment with *Toxocara* eggs (**Chapter 6**). The prevalence and risk factors for patent *T. canis* infections were defined in a cohort of 916 household dogs in the Netherlands (**Chapter 2**). In the same study, the owners' attitude towards deworming was assessed to elucidate whether the recognized zoonotic potential of *Toxocara* is an incentive for owners to deworm. The same was included in **Chapter 4** with respect to deworming of cats. Because dogs often show coprophagic behaviour, which may result in shedding of *Toxocara* eggs without actually having a patent infection, **Chapter 5** describes an investigation into the effect of coprophagic behaviour on the reported prevalence of patent *Toxocara* infections in household dogs. Subsequently, **Chapter 6** addresses the relative contribution of household dogs to the overall contamination of the environment with *Toxoxcara* eggs. It also evaluates what the effect of certain intervention strategies on this contribution might be. Finally, **Chapter 7**

focuses on both risk factors for a first observed patent infection as well as recurrent infections in a prospective study of the same cohort of dogs as used in **Chapter 2**. This final chapter aimed at the possibility to profile high-risk and low-risk dogs for having recurrent patent *Toxocara* infections. Results from all studies are discussed in view of providing possibilities and ideas to improve and rationalize control strategies to reduce the environmental contamination with *Toxocara* eggs by household dogs, beyond the currently recommended blind deworming strategy of at least four times a year.

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Chapter 2
Toxocara canis in household dogs: prevalence, risk factors and owners' attitude towards deworming

Parasitology Research (2015), 114: 561-569

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Illustratie: Wim Hendrikx
Toxocara II
lijnets/aquatint 2016

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 ≤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 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 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 ina 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 g/cm3). 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×.

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 *p* value 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 vari-

ables 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 ≥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, Results 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.

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

Helminths	*n*	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 %
Capillaria 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 %

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 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 ≤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 nonpredating 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,

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

Deworming frequency	All dogs			Non-coprophagic, unkenneled and leashed dogs[b]		
	n	*Toxocara*-positive dogs	*Toxocara* prevalence (95 % CI)	*n*	*Toxocara*-positive dogs	*Toxocara* 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)[a]
Four times a year	148	12	8.1 (4.3–13.7)	36	0	0.0 (0.0–9.7)[a]
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)[a]
Total	916	42	4.6 (3.3–6.2)	219	5	2.3 (0.7–5.3)

[a] *One-sided, 97.5 % confidence interval*

[b] *Leashed dogs are defined as dogs wandering off-leash less than 50 % of their walking time*

eight variables with a *p* value ≤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 ≤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 ≤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 *Toxocara*positive ($p<0.05$), 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 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

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[b]		
	n	*Toxocara*-positive dogs	*Toxocara* prevalence (95 % CI)	*n*	*Toxocara*-positive dogs	*Toxocara* prevalence (95 % CI)
≤1 month	100	8	8.0 (3.5–15.2)	21	0	0.0 (0.0–16.1)[a]
1–2 months	75	1	1.3 (0.0–7.2)	23	0	0.0 (0.0–14.8)[a]
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)

[a] *One-sided, 97.5 % confidence interval*

[b] *Leashed dogs are defined as dogs wandering off-leash less than 50 % of their walking time*

Table 4. Risk factors for shedding Toxocara *in dogs*

Variable	% exposed among *Toxocara*-positive dogs (*n*=42)	% exposed among *Toxocara*-negative dogs (*n*=874)	Single-variable OR[a] (95 % CI)	Multivariable OR[b] (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[c]	2.38	9.84	0.21 (0.03–1.57)	

[a] *Adjusted for age, coprophagy, except for the eponymous variables, and clustering of dogs living in the same households*

[b] *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*

[c] *Excluding dietary supplements (e.g. vitamins, minerals, etc.) and homeopathic compounds*

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

Deworming frequency per year		Reason for deworming				
		Dog's health	Public health	Combination[b]	Dogmatic[c]	Total
1x	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
2x	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
3x	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
4x	No	100	26[a]	11	8	145
	Row%	69.0	17.9[a]	7.6	5.5	100
	Column%	23.8	28.6[a]	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

[a] ESCCAP advised guidelines for standard blind deworming
[b] Reason for deworming was a combination of 'dog's health' and 'public health'
[c] The recognized reason for deworming by the owner was 'because we must'

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 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 freeranging 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.

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Chapter 3
Increase in number of helminth species from Dutch red foxes over a 35-year period

Parasites and Vectors (2014), 7: 166

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Illustratie: Wim Hendrikx
Toxocara III
lijnets/aquatint 2016

Background

The red fox (*Vulpes vulpes*) is host to a community of zoonotic and other helminth species. Tracking their community structure and dynamics over decades is one way to monitor the long term risk of parasitic infectious diseases relevant to public and veterinary health. We identified 17 helminth species from 136 foxes by mucosal scraping, centrifugal sedimentation / flotation and the washing and sieving technique. We applied rarefaction analysis to our samples and compared the resulting curve to the helminth community reported in literature 35 years ago. Fox helminth species significantly increased in number in the last 35 years (p-value <0.025). *Toxascaris leonina*, *Mesocestoides litteratus, Trichuris vulpis* and *Angiostrongylus vasorum* are four new veterinary-relevant species. The zoonotic fox tapeworm (*E. multilocularis*) was found outside the previously described endemic regions in the Netherlands.
Helminth fauna in Dutch red foxes increased in biodiversity over the last three decades.

Keywords: Helminth fauna, Red fox, Biodiversity, Molecular analysis, *Echinococcus, Toxocara, Taenia, Alaria*

Introduction

Long-term studies on parasite communities of marine and terrestrial wildlife hosts were instrumental to evaluating the influence of natural and anthropogenic factors on environmental changes, especially when sampling series span more than ten years [1-3].

For larger mammals, like the red fox, many cross-sectional studies report on the parasitic helminth fauna [4-13] or focus on limited parasite species [10, 12, 14-19], but long-term studies are rare [9].

In the 1980's, Borgsteede [4] studied the helminth fauna in foxes from the border region in the eastern part of The Netherlands, collected between February 1978 and May 1979. For ensuing decades, this study has been the sole large scale surveillance of helminth fauna in red foxes in the Netherlands.

A series of additional large scale surveillance in red foxes became reality since the initial detection of *Echinococcus multilocularis* in the Netherlands in 1996 [20]. *E. multilocularis* tends to increase in the fox population over the last decades in Europe [21] and therefore, the European Food Safety Authority (EFSA) recommends monitoring this parasite in foxes, especially at the borders of its distribution area in Europe [22]. Following the initial detection in the Netherlands, *E. multilocularis* in foxes was found to disperse in southern Limburg, but not in the central and western part of the Netherlands [20]. Since the Netherlands are a densely populated country with an average human population density of 497/km^2 [23] and a pet population of around 1.5 million dogs [24], a high density of red foxes (0.5 to 4.0 per square kilometre) might potentially lead to exposure of humans and dogs to zoonotic parasites, like *E. multilocularis* [16].

Here, we compared our recent large-scale surveillance of helminth fauna in the population of red foxes from the border region in the eastern part of The Netherlands with the historic studies more than 35 years ago. We evaluated trends in parasite richness by applying the rarefaction analysis [25, 26]. In addition, we discuss the relevance of our findings for public health.

Materials and methods

Animals
From October 2010 until April 2012, routinely shot foxes were collected by hunters and sent to the National Institute for Public Health and Environment (RIVM,

Bilthoven, The Netherlands). The chosen fox sample size (288) originated from a strip with a width of 15 km and a length of 266 km at the border with Germany, between Groningen and Limburg (4000 km^2), excluding the formerly found positive districts (Figure 1). Upon arrival, fox carcasses were stored at -80˚C to inactivate the eggs of *E. multilocularis* [27], according to WHO guidelines [28]. After a minimum period at -80˚C of one week, carcasses were thawed and dissected. Data on weight, measurements, age and gender were collected after thawing. From weight and body size, condition was estimated as the ratio of body weight in gram over body length (nose-anus) in millimetres (body weight / length index, BWL).

Figure 1. Geographical origin of individual foxes. This figure shows the study area along the eastern border of the Netherlands in blue, with a representation of the whole country in black. Circles show the geographical origin of the foxes collected for this study.

The age of the foxes was evaluated by examining tooth wear, especially the wear of the lower incisors and the upper and lower molars and by cutting the root of one or two canines into several 0.15 mm thin slices which were examined microscopically (magnification 20-40 times) under horizontal cross light [29]. Foxes without signs of wear were classified as first year animals [30].

During dissection, the jejunum and faecal material (if present) from the distal colon/rectum of each fox were sampled. The whole small intestines of 262 foxes were evaluated by microscopic examination of mucosal scrapings and macroscopic examination of the opened small intestine. Moreover, distal colon content was used for PCR (see *E. multilocularis*-specific PCR identification); 158 foxes had sufficient faecal content in the colon to be used for additional microscopic analysis after centrifugal sedimentation / flotation.

Microscopical examination of parasites

Small intestine mucosal scraping
The small intestine of each fox was separated and opened. Macroscopically visible helminths were scored and noted. Subsequently, mucosal scrapings were made to screen the mucosal content for small helminths microscopically [31, 32]. The presence of intestinal helminths was scored semi-quantitatively: '+' 1-2 individuals, '++' 3-10, '+++' 11-50, '++++' 51-100 and '+++++' >100. Parasites were identified morphometrically and in cases where difficult to identify young adult stages were found, or the freezing/thawing process had damaged the morphology of cestode species, morphological identification was confirmed by PCR (see Molecular identification of parasites). For this purpose, parasite specimens were collected and stored in 70% ethanol until further use.

Sedimentation / flotation on gut content
When available, about 3 grams of distal colon content were suspended in 50 ml tap water, an 11 ml centrifuge tube was filled with this suspension and the product of centrifugal sedimentation / flotation was examined microscopically. A sucrose solution of 1.28-1.3 g/cm^3 was used as flotation medium for the faecal examination of eggs and larvae. The centrifugal step for flotation was performed with the cover slip on top of the tube and one slide was examined per sample. The results were scored semi quantitatively using '+' for 1-10 eggs per slide; higher numbers were scored as '++' for one to five per microscopic field at 100x (10x10) magnification and '+++' for more than five per microscopic field at the same magnification.

Since fox carcasses were frozen to inactivate zoonotic parasites, the Baermann method could not be used to isolate first stage larvae of *Crenosoma vulpis* and *Angiostrongylus vasorum*. Larvae that were found by CSF, which were not too damaged by the freezing and thawing process were identified morphologically according to McGarry and Morgan (2009) [33].

Screening for cardio-pulmonary helminths
The lungs and hearts of 97 foxes were examined for helminths by opening the right heart and pulmonary arteries up to the level of small branches in the lungs [34]. The bronchi were opened, examined and washed with water, which was sieved through a 150 µm mesh size sieve. The same procedure was used for heart and vessels. Adult and juvenile worms were removed from the sieve and identified morphologically up to species level [35, 36].

Screening for helminths in the urinary bladder
In addition, four urinary bladders were opened to look for adult worms of *Pearsonema plica*.

Helminth species number
To evaluate a possible change in helminth species richness, we applied rarefaction analysis [25, 26] to the number of distinct helminth species that we identified in 136 foxes. We calculated the rarefaction curve with the software package EstimateS 9.0 [25, 26, 37] with default settings. Based on the rarefaction curve, we compared our findings with those of historical studies [4-6, 8, 9].

Foxes, for which biological parameters or geographical data were missing, were excluded from analysis. This limited the available dataset for multifactorial analysis to 136 foxes. For each parasite species, prevalence was calculated and significance of prevalence difference was analyzed with Fisher's Exact test. Correlations between body condition, age, gender and parasite prevalence were determined by ANOVA (analysis of variance). Fisher's exact test and ANOVA were performed and the resulting P-values were calculated using Quickcalc (GraphPad Software, Inc. La Jolla, California, USA) and the data analysis module of Microsoft Excel 2007.

E. multilocularis-*specific PCR identification*
To analyse the presence of *E. multilocularis* at sub-microscopical level, three grams of colon contents were tested in a single tube nested 12S ribosomal DNA PCR as described previously [20]. PCR products were specified by southern blot hybridization, using *E. multilocularis*- specific probes as described previously [38].

Molecular identification of parasites

DNA isolation and PCR
Parasites were transferred from 70% ethanol and soaked in demineralized water. DNA was isolated using the Qiagen Blood and Tissue Kit (Qiagen NV, Venlo, The Netherlands), according to the manufacturer's instructions. To confirm the identification of cestode species, a fragment of the mitochondrial cytochrome oxidase 1 (CO1) gene was amplified as described by Bowles et al. [39]. All PCRs were carried out in 50 µl final volume containing 3 µl genomic DNA, 0.5 µl of each forward and reverse primer (50 µM stock) and 25 µl of Qiagen HotstarTaq polymerase master mix (Qiagen NV, Venlo, The Netherlands). The final reaction volume was adjusted to 50 µl with sterile demineralized water. PCR amplification of the partial CO1 gene was performed using the following conditions: denaturation at 95 °C for 15 min, followed by 35

cycles of 1 min denaturation at 95 °C, 1 min annealing at 45 °C, 1:15 min elongation at 72 °C, followed by a final extension step of 7 min at 72 °C.

DNA sequencing of amplicons
PCR amplicons were purified using standard procedures (ExoSAP-IT®, Affymetrix, Cleveland, Ohio, USA). All DNA sequence PCR reactions were carried out on both DNA strands in 20 µl final volume containing 3 µl of amplicate, 7 µl sequence buffer, 1 µl of Big Dye Terminator and 1 µl of each PCR primer. Sequence PCR was performed under the following conditions: 95 °C for 1min, followed by 25 cycles of 96 °C for 10 min, 50 °C for 5 min and finally 60 °C for 4 min. Trace files of the obtained sequences were generated on an automated ABI sequencer at the Institute's DNA sequence facility.

DNA and phylogenetic analysis
DNA sequences were assembled, edited, and analysed with BioNumerics version 6.6 (Applied Maths NV, Sint-Martens-Latem, Belgium). Obtained CO1 gene sequences were compared to reference sequences present in Genbank after subtraction of the primer sequences. Cluster analysis of the sequences was conducted using the unweighted neighbour-joining algorithm of the BioNumerics program. Bootstrap proportions were calculated by the analysis of 2500 replicates for neighbour-joining trees. Available CO1 sequences of cestodes and trematodes from Genbank were included in the alignment. Sequence homology ≥99% and homology of morphological criteria were considered as proof of identity between isolated and Genbank species. Unequivocally identified *Alaria alata* isolates from foxes from this study served as out-group in phylogenetic analysis.

Results

Animal age, gender and body weight
In total, 262 foxes were collected. Seventy per cent of the foxes were 7-12 months old at the time of sampling and seven foxes were older than 5 years. This age distribution of shot foxes indicates high hunting pressure as found in previous studies [30, 40].

Overall, 55% of the sampled foxes were males and 45% were females, which were evenly distributed over the study area (Figure 1). Males were heavier than females; average body weight / length (BWL) index of males and females differed significantly (ANOVA, P-value < 0.0001). Correlation between BWL index and infection classes was absent for both male foxes (P-value = 0.626) and female (P-value = 0.232).

Analysis of helminth species number

Seventeen helminth species were identified from our reference data set of 136 foxes. The 95% confidence interval was 14.39 – 19.61 parasite species. The number of parasite species in 137 foxes that were sampled 35 years ago [4] was twelve species, which is a significantly lower species richness (P-value < 0.025) (Figure 2).

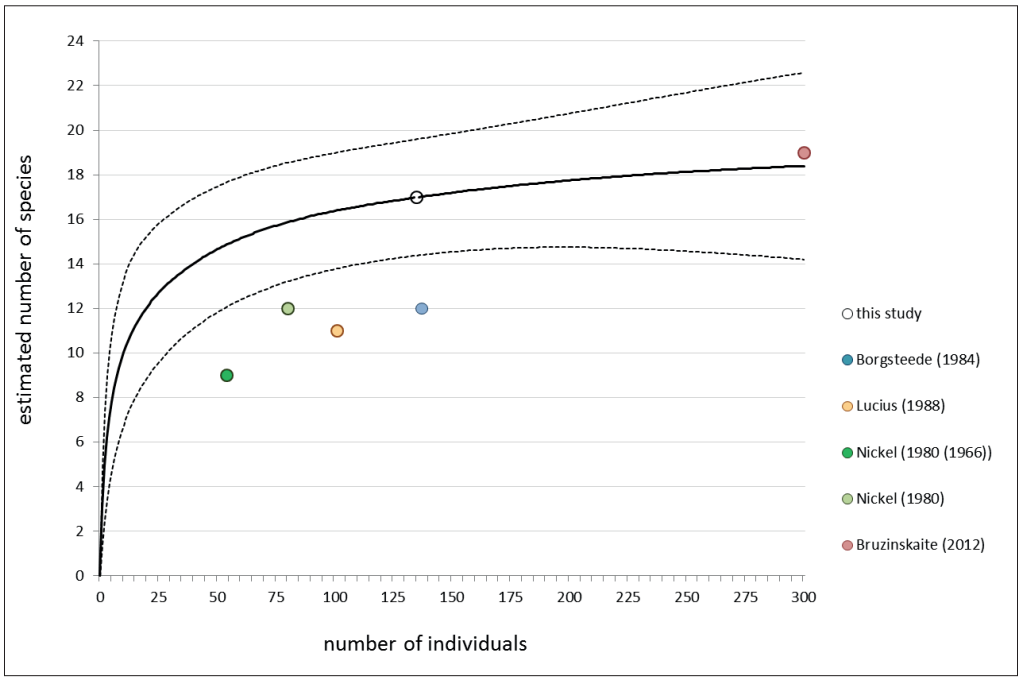

Figure 2. Analysis of fox parasite species by rarefaction method.
Open circle: the number of distinct parasite species identified from 136 Dutch foxes in this study. Solid circle: the number of distinct parasite species identified from the foxes described in a cited study. Solid line: expected number of distinct parasite species estimated by the rarefaction method based on our data set (i.e. open circle). Dotted line: 95% confidence interval. Nickel et al. [9] reported two independent fox populations from different regions, sampled in 1966 (green solid circle) and in 1980 (light green solid circle) respectively.

Multiple infections per fox

On average 97.1% of the foxes were infected with one or more out of 17 helminth species, with maximum co-infection levels of eight different species.
Foxes younger than 10 months were more frequently infected (35-37%) with 2-3 parasite species than foxes older than 10 months (10-27%) (Figure 3).

Prevalence per helminth species and comparison with other studies

Parasite prevalence was higher in male foxes for the majority of the parasite species

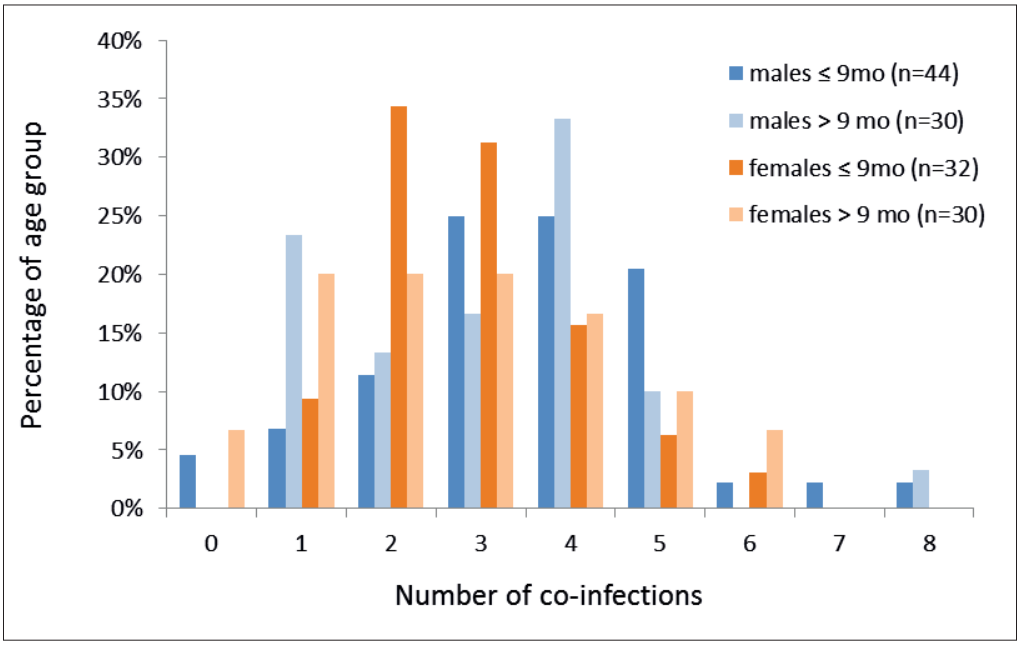

Figure 3. Number of co-infections per age group and per gender.
Male foxes peak at three to four co-infections, females nine months of age and younger peak at two to three co-infections. Male foxes exhibit the highest numbers of co-infection (8). Zero co-infections mean no infection at all. Total number of foxes is 136.

(Table 1), although this was only significant for *Toxocara canis* (Fisher's Exact test, P=0.013). *T. canis* and *U. stenocephala* were the most prevalent intestinal fox parasites in our study, like in other Western European countries [5-7, 14-16]. The prevalences of *T. canis* and *Taenia* spp. were significantly lower in this study compared to the earlier study of Borgsteede [4] (Table 2).

The combined prevalence of *Toxocara canis* and *Toxascaris leonina* reported in Belgian foxes in 2005 [16] was not different (Fisher's Exact test, P=0.315) from the prevalence in our study. The prevalence of *T. canis* in Danish foxes in 2006 [6] was 59.4%, which is almost identical to the level found in this present study, as was the case for *Taenia* species. In contrast, the prevalence of *Uncinaria stenocephala* was significantly higher in Denmark [6], compared to either our data (Fisher's Exact test, P=0.0018), historical data from northern Germany [5] (Fisher's Exact test, P=0.002), or historical data from the Netherlands [4] (Fisher's Exact test, P=0.054).
The prevalences of *Strongyloides* sp., *Eucoleus aerophilus* and *Crenosoma vulpis* was significantly higher than reported in 1984 [4] (Table 2). *Trichuris vulpis*, *Angiostrongylus vasorum*, *Mesocestoides litteratus* and *Echinococcus multilocularis* were new spe-

Table 1. Overview of parasitic helminths found in Dutch red fox.

	males (n=73)		females (n=63)		overall (n=136)		Means of infection	Method
Intestinal nematodes	%	n	%	n	%	n		
1. *Toxocara canis*[1]	71.2	52	49.2	31	61.0	83	worm eggs, paratenic hosts	D, CSF
2. *Toxascaris leonina*	1.4	1	3.2	2	2.2	3	worm eggs, paratenic hosts	CSF
3. *Trichuris vulpis*[2]	20.5	15	12.7	8	16.9	23	worm eggs	CSF
4. *Uncinaria stenocephala*	60.3	44	47.6	30	54.4	74	free larvae, paratenic hots	CSF, MS
5. *Strongyloides* sp.	9.6	7	20.6	13	14.7	20	free larvae	CSF, MS
Other nematodes								
6. *Eucoleus aerophilus* (n=96)	71.4	35	63.8	30	67.7	65	earthworms, worm eggs	WS
7. *Pearsonema plica*	(2/2)		(2/2)		(4/4)*		worm eggs	D. WS
8. *Capillaria* spp.[3]	52.1	38	47.6	30	50.0	68	worm eggs	CSF
9. *Angiostrongylus vasorum* (n=96)	6.1	3	2.1	1	4.2	4	terrestrial gastropods, frogs	WS, CSF
10. *Crenosoma vulpis* (n=96)	24.5	12	8.5	4	16.7	16	terrestrial gastropods	WS, CSF
Intestinal cestodes								
11. *Taenia crassiceps* / 12. *Taenia polyacantha*	21.9	16	22.2	14	22.1	30	rodents, lagomorpha	D, MS, PCR
13. *Mesocestoides litteratus*	6.8	5	4.8	3	5.9	8	frogs, intermediate hosts	D, MS, PCR
14. *Echinococcus multilocularis*	1.4	1	0.0	0	0.7	1	rodents, lagomorpha	PCR
Intestinal trematodes								
15. *Cryptocotyle lingua*	4.1	3	3.2	2	3.7	5	fish	MS
16. *Isthmiophora melis*	1.4	1	0.0	0	0.7	1	tadpoles	MS
17. *Alaria alata*	17.8	13	15.9	10	16.9	23	tadpoles, frogs	MS, PCR

[1] The observed prevalence in *T. canis* between male and female foxes is significantly different (Fisher's Exact test, P=0.013). [2]This diagnosis was not confirmed by demonstrating adult worms in the colon. [3]*Capillaria* spp. eggs were not identified to species level due to morphological changes as a result of freezing and thawing. Methods used for detection and speciation. D: dissection, CSF: centrifugal sedimentation / flotation, MS: mucosal scraping, WS: washing and sieving. Species number 6, 9 and 10 were obtained from heart and lung washings for which 96 foxes were available. *: Four out of four urine bladders were found positive for this species, but prevalence was not extrapolated from this limited number of analyses.

Table 2. Parasite prevalence in red fox compared to 35 years ago.

	Zoonotic species	Netherlands Borgsteede (1984) (n=137)	Netherlands This study (n=136)	Fisher Exact P (2-sided)
Intestinal nematodes		%	%	
Toxocara canis	Yes	73.7	61.0	0.028
Toxascaris leonina	No	0	2.2	0.122
Trichuris sp.	No	0	16.9	<0.0001
Uncinaria stenocephala	Yes	59.9	54.4	0.393
Strongyloides sp.	Yes[1]	0.7	14.7	<0.0001
other nematodes				
Eucoleus aerophilus	No	46.8	67.7	0.285
Pearsonema plica	No	23.5	(4/4)[2]	-
Capillaria spp.			50.0	-
Angiostrongylus vasorum adults/larvae	No	(0)[3]	4.2	0.028
Crenosoma vulpis adults/larvae	No	4.5	16.7	0.008
Cestodes				
Taenia spp.[4]	Yes[5]	53.3	22.1	<0.0001
Mesocestoides sp.	No	0	5.9	0.003
Echinococcus multilocularis	Yes	0	0.7	0.498
Trematodes				
Cryptocotyle lingua	No	3.6	3.7	1
Eupariphium melis	No	1.5	0.7	1
Alaria alata	No	10.9	16.9	0.166
Opistorchis felineus	Yes	0	0	-
Apophallus donicus	No	0.7	0	0.498
noninfected (over-all)		2.9	2.9	

Differences between this study and the Borgsteede study (1984) are indicated (Fisher's exact test). [1] *Strongyloides* species are non-zoonotic, whereas *S. stercoralis* is infectious to humans and is a species of warm geographical zones, although found in a dog kennel in Finland [55]. [2] This species was present in four analysed urinary bladders, therefore prevalence difference was not analysed. [3] The first documented cases of autochthonous French heartworm were seen in 2009. [4] Data on *Taenia* species were combined to facilitate comparison with other studies. [5] In our study, *T. crassiceps* and *T. polyacantha* were found, the former of which is zoonotic.

cies in de studied area. The trematode *Apophallus donicus*, of which one individual was found by Borgsteede [4] was not identified in the present study. This was also the case for *Hymenolepis* spp., for which rodents are definitive hosts. Adult *Hymenolepids* are regarded as passing species from prey, as is *Molineus patens*, and these were thus excluded from analysis of helminth species parasitic to red fox.

E. Multilocularis-*specific PCR identification*
All foxes were negative for this species by microscopical examination of mucosal scrapings, but one fox out of 262 investigated foxes was positive for *E. multilocularis* (prevalence 0.7%; 95%CI 0.02-2.1%), using the 12S single tube nested PCR and subsequent southern blot analysis on faecal content. This positive result was confirmed after repeated testing of the fecal content. Up to this study, no positive foxes were identified in the presently studied area.

Molecular characterisation of intestinal parasites
PCR products of *Taenia polyacantha*, *Taenia crassiceps* and *Alaria alata* were all 403 bp in length. These DNA sequences were submitted to Genbank [accession numbers KF751222-KF751223 (*T. crassiceps*, isolates V1382 and V1336), KF751225-KF751226 (*T. polyacantha*, V1361 and V1269) and KF751233-KF751234 (*A. alata*, V1338 and V1359)].

Microscopic identification of cestodes was confirmed by cluster analysis of the partial CO1 gene sequences. The inferred Neighbour Joining tree shows very high homology between obtained CO1 sequences and Genbank entries for *T. crassiceps* from Russia and Norway (EU544549), *T. polyacantha* from Denmark and Finland (EU544583, EU544584, EU544585 and EU544586) and for the trematode *A. alata* from Lithuania and Germany (HM022221, HN022222 and HM022224), the latter of which served as outgroup (Figure 4).

Discussion

This study shows an increased diversity in the helminth parasite community of Dutch red foxes compared to a study conducted in the same region 35 years ago [4]. We report four new records of veterinary importance: *Toxascaris leonina*, *Mesocestoides litteratus, Trichuris vulpis* and *Angiostrongylus vasorum*. The finding of a fifth (zoonotic) species –*Echinococcus multilocularis*– has been described earlier for the Netherlands [20], but not in this same geographical area.

We used a combination of microscopic and molecular techniques to evaluate the helminth fauna of red fox as described above, whereas Borgsteede (1984) and Lucius et

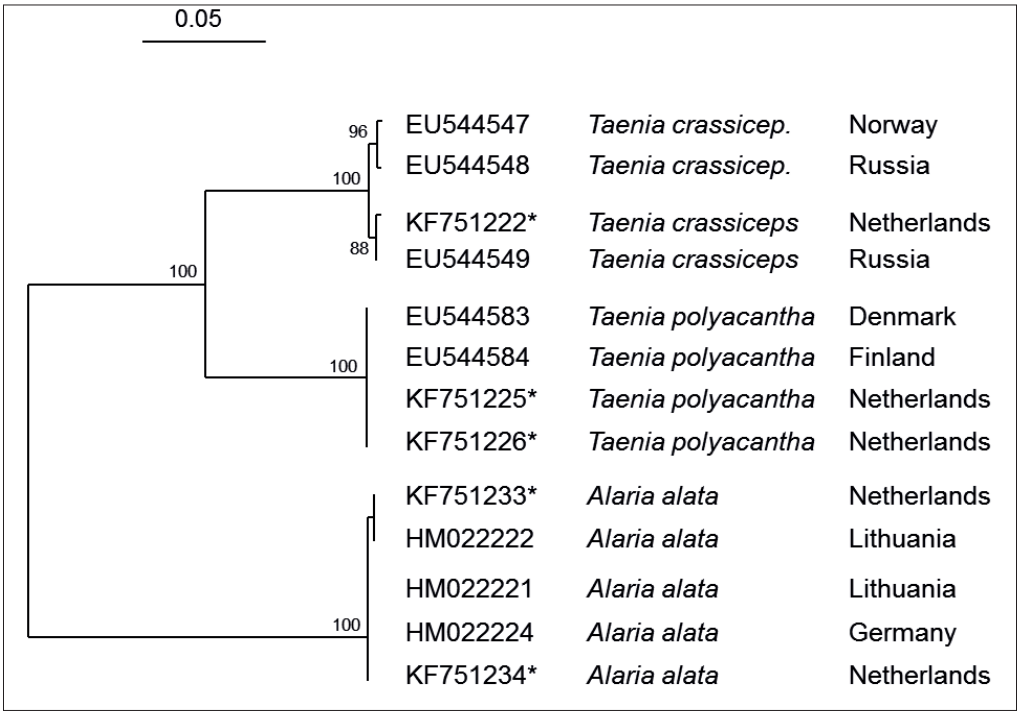

Figure 4. CO1 Neighbour Joining Tree of European fox cestode isolates.
Taenia *species found in red fox (* this study) show high homology with other European isolates found in Genbank (bootstrap values of 2500 simulations).* Alaria alata *is used as outgroup and here too, the Dutch isolates show high homology with other European isolates from Genbank. Bar indicates base substitutions per site.*

al. (1988) used microscopy following the washing and sieving technique. Use of the more sensitive PCR technique in this present study might have biased the observed biodiversity to some extent, since it was not available in the period of the study of Borgsteede, but this does not explain the observed biodiversity increase compared to older studies. Confirmation of the identity of cestode species that had been found microscopically by PCR in this present study, did not lead to more cestode species compared to historic data. Moreover, even without *E. multilocularis*, which was demonstrated only by PCR, significantly more helminth species were found in this present study, compared to historical data (result not shown). The introduction of *E. multilocularis* and *A. vasorum* into the Netherlands is documented [20, 38, 41]; these independent studies support the increased biodiversity of helminth fauna in the population of red foxes in the Netherlands. The study of van der Giessen et al (1999) [20], for which a combination of mucosal scraping and PCR was used, demonstrated

presence of *E. multilocularis* in the eastern border region, both north and south to the present study area, but not in the latter, which was included in that study as well. This finding confirmed the observation of Borgsteede [4] at that time.

Parasites indicated as *Capillaria* spp. might include more fox specific species, like *Eucoleus boehmi*, which is endemic to the Netherlands (H. Cremers, unpublished data), and other species passing through the gut after predation; however these were not further identified to species level.

Rarefaction and extrapolation of parasite richness and abundance data (this study) revealed a significant increase of species richness compared to 12 different fox parasite species determined by Borgsteede [4], 11 species found by Lucius et al (1988) [5] and 9-12 species found in two regions of the former German Democratic Republic respectively in 1966 and in 1980 [9]. Recent studies in the Northern European hemisphere [6, 8] show species richness that fits the asymptotic maximum of the estimated species richness calculated from our data. This increase might be driven by a combination of natural developments and or anthropogenic causes (global warming, climatic fluctuations). It is however, beyond the scope of this paper to identify the drivers for the observed increase in the parasite biodiversity.

Parasites of veterinary importance may be introduced into the environment through pet travel or translocation of wildlife hosts. *Angiostrongylus vasorum* only recently became endemic to the Netherlands [41] and is known for its endemic foci in Dutch dogs [41]. In the present study, we found *A. vasorum*-positive foxes in the southern half of the study area, outside and distant from the published endemic foci, which demonstrates a wider endemic area sustained by the red fox.

In this study, *E. multilocularis* parasite DNA was identified by PCR in the intestinal content of one red fox in the northern part of the Dutch-German border area. The identification based solely on molecular techniques suggests a very low intestinal abundance in the infected fox, well below the detection level of microscopy. Previous studies showed PCR to be more sensitive, compared to the mucosal scraping method, especially at low endemicity [20, 42].

The observed *T. canis* prevalence decline in foxes (-17%) is also recognised in the human population, since data from a Dutch cohort study show a moderate but significant decrease of *T. canis* exposure between 1998 and 2004 [43]. However, this is not recognised in prevalence of patent infections in dogs [44-47].
The prevalence of *Taenia* spp. showed the sharpest decline (-59%), followed by *T. canis* (-17%), compared to the study by Borgsteede [4]. Among fox prey are rodents,

which are obligate intermediate hosts in the lifecycle of cestode parasites like *E. multilocularis* and *Taenia* spp., and facultative intermediate hosts of nematodes like *T. canis.* Small mammals, especially voles (*Microtus arvalis* and *Arvicola terrestris*), comprise almost 50 % of the fox's prey during autumn and winter [30, 48, 49]. The decreasing prevalence of *Taenia* spp. and *T. canis* in foxes might be correlated with the decreasing abundance of rodents [50, 51], which is also indicated by decline of raptor species exclusively preying on rodents [52, 53].

We were able to identify *Taenia crassiceps* and *T. polyacantha* from frozen material, using morphological data in combination with molecular techniques. A combination of detection techniques as presented in this study might be useful to increase sensitivity and specificity and to differentiate host-specific parasites from parasite eggs and/or larvae passing after ingestion of prey. CO1 gene sequences of *A. alata*, *T. crassiceps* and *T. polyacantha* from Dutch fox (this study) were homologous with isolates from European countries at the North or East of the Netherlands (Germany, Denmark, Lithuania, Finland and Russia). Previously, spatial prevalence analysis across borders demonstrated radiation of *E. multilocularis,* from the adjacent Belgian fox population to the southern Dutch fox population [20, 54].

In conclusion, we infer a significant increase in parasitic helminths diversity in the fox population at the eastern border of the Netherlands over a period of 35 years. In the same period, the prevalence of two zoonotic helminths species belonging to different genera declined. In addition, four veterinary-important species were identified for the first time in this present study, and three additional species showed higher prevalence over that period. We identified the fox tapeworm *E. multilocularis* for the first time outside the previously described endemic spots in the Netherlands. Due to the very low prevalence and abundance, the infection risk for humans in the studied area is considered limited. It remains important, however, to follow the spread of *E. multilocularis* in this area in the future.

Competing interests
The authors declare that they do not have competing interests.

Authors' contributions
FF generated and analysed parasitological data, performed molecular lab work and sequence analysis, and wrote the manuscript, RN generated parasitological data and wrote the manuscript, JM generated biological data concerning the collected foxes, HC generated parasitological data concerning non-intestinal helminths, CD did the molecular lab work concerning *E. multilocularis*, KT wrote the study design and manuscript, and helped with statistical analysis, JvdG wrote the study design,

conceived and wrote the project proposal, coordinated the study, generated parasitological data and contributed to the manuscript. All authors read and approved the final manuscript.

Acknowledgements
This study was partially financed by the Dutch Food Safety Organisation (NVWA). The authors thank Merel Langelaar, Marieke Opsteegh and Manoj Fonville for their valuable contribution to the study coordination, dissection of foxes and the parasitological examinations. We are also thankful to Margriet Montizaan of the Dutch Hunters Association (KNJV) and hunters for providing foxes.

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Chapter 4
Prevalence and risk factors for patent *Toxocara* infections in cats and cat owners' attitude towards deworming

Parasitology Research, In press

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Illustratie: Wim Hendrikx
Toxocara IV
lijnets/aquatint 2016

Abstract

The prevalence of and risk factors for shedding *Toxocara* eggs in cats older than 6 months were determined by examining 670 faecal samples collected in 4 cross-sectional studies in the Netherlands. Additionally, cat owners provided information on their attitude towards routine deworming. Samples were examined using the centrifugal sedimentation flotation method. Overall *Toxocara* prevalence was 7.2%. Multivariable logistic regression analysis revealed that young age and living in rural areas were significant risk factors for shedding *Toxocara* eggs. Moreover, the more time a cat was allowed to roam outdoors the higher was its risk to shed *Toxocara* as compared to cats with no outdoor access at all. For 199 cats (81.6% of cats subjected to a deworming regimen) owners provided the reason for treatment. The main reason for routine deworming (80.4%) concerned the cat's health and only 10.6% of the cats were treated for public health reasons. Moreover, the generally advocated four-times-a-year deworming advice was applied on only 24.5% of cats. We concluded that free-roaming is a key factor in the acquisition of patent *Toxocara* infections which leads to the environmental contamination with *Toxocara* eggs. Additionally, the knowledge of cat owners is still insufficient to expect them to make sound decisions on routine deworming.

Keywords: *Toxocara cati*, Household cats, Risk factors, Deworming, Cat owners, Public health

Introduction

Cats are among the most common pets worldwide, and in a country like the Netherlands, their estimated number is almost twice as large as that of dogs (HAS den Bosch and Utrecht University 2015). Additionally, while the Netherlands is a country free of stray dogs, stray and free-ranging cats are widespread (Neijenhuis and van Niekerk 2015). These unowned cats are more likely to receive sub-optimal care and potentially harbour more parasites.

Toxocara cati is a zoonotic roundworm of cats that is known to commonly affect both well-cared and stray cats. Compared to its congeneric species *Toxocara canis* in dogs, the epidemiology of *T. cati* is more unclear (Fisher 2003). However, among adult hosts of *Toxocara* spp. in the Netherlands, i.e. cats, dogs and foxes, cats have been estimated to be responsible for a considerable, if not the largest, portion of *Toxocara* spp. eggs contaminating the environment (Morgan et al. 2013; Nijsse et al. 2015). With the aim of reducing environmental contamination with *Toxocara* eggs, the guidelines of the European Scientific Counsel Companion Animal Parasites (ESCCAP) state that all adult cats should be dewormed at least four times a year to prevent patent *T. cati* infections (ESCCAP September 2010). However, the compliance of cat owners to this advice is unlikely to be high enough to have a significant impact on the environmental contamination with *Toxocara* eggs (Overgaauw et al. 2009).

The prevalence of patent *Toxocara* infections in adult cats is assumed to be higher than that in adult dogs (Overgaauw 1997; Fisher 2003; Michalczyk and Sokol 2008; Gates and Nolan 2009; Overgaauw et al. 2009). Nevertheless, like in household dogs, most of the household cats are unlikely to shed *Toxocara* eggs at the moment of being dewormed blindly, i.e. without laboratory confirmation of *Toxocara* infection. *Toxocara* prevalence rates in cats vary from 2 to 79%, depending on the country, diagnostic test, and population under study (e.g. indoor household cats, household cats with outdoor access, stray cats, sheltered cats, etc.) (Engbaek et al. 1984; Overgaauw and Boersema 1998). By burying their faeces, cats can contaminate the environment more than just superficially. Sandpits in children's playgrounds appear to be one of the preferred spots for free-ranging cats in urban areas to defaecate, posing children at high risk of infection (Uga et al. 1996). Therefore, cats deserve more attention as a likely source of human toxocariasis (Fisher 2003).

The aim of this study was to determine the prevalence and risk factors for shedding *Toxocara* eggs in cats. Additionally, we assessed the attitudes of cat owners towards deworming.

Material and methods

In total, 670 faecal samples from cats were coproscopically examined. These samples came from privately owned cats (n=353) and from cats that were recently brought to an animal shelter (n=317). Cat owners and animal handlers in the shelters participated voluntarily. Of the sheltered cats, 95 had a history of straying and 20 were recently abandoned; for 202 sheltered cats no history was provided. Parasitological examination of faecal samples was combined with epidemiological data collection using questionnaires. Faecal samples were collected during four different periods within the frameworks of four different cross-sectional studies on feline parasites: 1) from October 2010 to January 2011; 2) from June to August 2014; 3) from April to May in 2015; 4) January to March 2016. The samples were either sent to the laboratory by the owners or by veterinarians working in a shelter or directly collected at the animal shelter by veterinary students. Every sample was processed within four days after defaecation.
At least 3 grams faeces per sample were examined at the parasitology laboratory of the Faculty of Veterinary Medicine of Utrecht University using the centrifugation sedimentation flotation technique. The amount of faeces was suspended in 55 ml of water and 11 ml of this suspension was used for centrifugal sedimentation followed by flotation using a sucrose solution with a specific gravity of 1.27-1.30 g/cm^3.

Questionnaires were answered online using Surveymonkey®. Owners needed to complete the questionnaires to obtain the results of the coproscopical examination. Because of the different purposes of the four studies, not every question was included in the questionnaire of all studies. A copy of the questionnaire is available on request to the authors. For the sheltered cats, the animal handlers were interviewed at the animal shelter or questions were handed in paper form and returned with the samples.

Data analysis
We assessed the association of 21 variables with positivity for *Toxocara* eggs using logistic regression models incorporating two-way cluster-robust standard errors as performed elsewhere (de Man et al. 2016) to account for clustering, i.e. non-independence, of cats at both the study (*n*=4) and household/shelter (*n*=395) levels. Variables showing p≤0.10 for the association with *Toxocara* positivity in the univariable analysis were selected for inclusion in a multivariable logistic regression model built in backward stepwise fashion to retain only those variables significantly associated (p<0.05) with *Toxocara* positivity. However, variables producing a change of ≥10% in the coefficients of the other covariates when removed from the models were retained regardless of their significance. Associations were expressed as odds ratios

(ORs) with corresponding 95% confidence intervals (CIs).
Collinearities between variables were checked before multivariable analysis and choosing between collinear variables was based on the improvement in model fit (Akaike information criterion) or on biological plausibility, reliability and number of observations when the collinear variables measured similar factors (Dohoo et al. 2009). Because of the limited number of outcome events, the final multivariable model was cross-validated by calculating bias-corrected bootstrap 95% CIs (1000 replications) to ensure that they did not differ significantly from the standard ones, as suggested elsewhere (Vittinghoff and McCulloch 2007; Nemes et al. 2009). Statistical analysis was performed using Stata v. 13 (StataCorp., USA).

Results

Prevalence of gastrointestinal parasites
Of the 670 faecal samples examined for all types of helminth eggs (Table 1), 54 were found positive for at least one type of helminth egg (8.1%, 95% CI: 6.2–10.5 %). In 49 cats, only one type of eggs was found, while 5 cats had a double infection. The most frequently found egg type was that of *Toxocara* sp. with a prevalence of 7.2% (95%CI: 5.4-9.4%). As the main focus of this study was on *Toxocara*, further results were presented for this specific helminth only.

Table 1. Prevalence of the different helminth egg types recovered at examination of cats' faeces.

Helminths	Positive cats (*n*=670 tested cats)	Prevalence	95% CI[1]
Toxocara sp.	48	7.2%	5.4-9.4%
Capillaria sp.	3	0.5%	0.1-1.9%
Taeniidae	7	1.1%	0.5-2.2%
Toxascaris leonina	0	0.0%	0.0-0.6%[2]
Hookworms	1	0.2%	0.0-1.1%
Dipylidium caninum	0	0.0%	0.0-0.6%[2]

CI = confidence interval
[1]Adjusted for clustering at the levels of study cohort and household
[2]One-sided, 97.5% confidence interval

Risk factors

The results of the univariable and multivariable analyses of the factors associated with positivity to *Toxocara* are reported in Table 2. Of the 12 factors showing a p≤0.10 in the univariable analysis that were selected for inclusion in the multivariable model, only 3 were significantly associated with *Toxocara* positivity in the multivariable analysis. These were cats' age group, average daily time spent outside, and living in rural areas. Specifically, compared to cats of ≤1 year of age, those aged 2-5 years and those aged ≥6 years had a decreased risk of being *Toxocara* positive (ORs 0.40 and 0.11, respectively). Conversely, the risk of being positive to *Toxocara* increased with the average duration of (unsupervised) outdoor time. Compared to cats that have, according to the owner, no outdoor access at all, an increased risk was found in those staying outside for an average of ≤1 hour/day (OR 2.02), 2-5 hours/day (OR 7.26), or ≥6 hours/day (OR 8.49). Finally, cats living in rural areas were at increased risk of being *Toxocara* positive (OR 7.48).

Table 2. Factors associated with increased or decreased odds for positivity to Toxocara *eggs in cats.*

Factor	n	*Toxocara* prevalence %[1]	Univariable OR[1]	Multivariable OR[1,2]
Age group				
≤1 year	36	19.4 (10-34.4)	Ref.	Ref.
2-5 years	321	7.8 (5.2-11.4)	**0.35 (0.15-0.80)[‡]**	**0.40 (0.26-0.64)[§]**
≥6 years	241	3.3 (1.7-6.4)	**0.14 (0.05-0.43)[§]**	**0.11 (0.10-0.12)[§]**
Unknown	72	11.1 (4.8-23.5)	0.52 (0.17-1.57)	0.26 (0.07-1.01)*
Gender				
Female	311	7.7 (5.3-11.2)	Ref.	
Male	336	6.8 (4.5-10.2)	0.88 (0.38-2.02)	
Unknown	23	4.3 (0.5-28.8)	0.54 (0.05-5.96)	
Time since last deworming				
≤1 month	80	8.8 (3.7-19.2)	Ref.	
2-3 months	160	5 (2.5-9.7)	0.55 (0.11-2.67)	
4-6 months	95	6.3 (2.8-13.6)	0.7 (0.15-3.32)	
≥7 months	129	2.3 (0.7-7.1)	**0.25 (0.07-0.86)[†]**	
Unknown	206	11.7 (7.7-17.3)	1.38 (0.62-3.05)	
Applied deworming regimen				
None	91	1.1 (0.2-7.6)	Ref.	
1x/year	42	0.0 (0.0-8.4)[4]	Not estimable	
2-3x/year	120	2.5 (0.8-7.6)	2.31 (0.22-23.7)	
≥4x/year	82	1.2 (0.2-7.8)	1.11 (0.07-17.68)	

Factor	n	*Toxocara* prevalence %[1]	Univariable OR[1]	Multivariable OR[1,2]
Unknown	335	12.8 (9.4-17.3)	**13.25 (1.75-100.12)[†]**	
Sterilization				
No	283	12.7 (9-17.6)	Ref.	
Yes	364	3 (1.7-5.3)	**0.21 (0.14-0.33)[§]**	
Unknown	23	4.3 (0.5-28.8)	0.31 (0.05-2.06)	
Outdoor access				
No	254	2.4 (1.1-5.1)	Ref.	
Yes	297	8.1 (5.4-12)	**3.63 (2.15-6.15)[§]**	
Unknown	119	15.1 (9.3-23.6)	**7.37 (1.58-34.41)[‡]**	
Average daily time spent outdoor				
None (no outdoor access)	254	2.4 (1.1-5.1)	Ref.	Ref.
≤1 hour	28	7.1 (1-37.4)	3.18 (0.87-11.66)*	**2.02 (1.08-3.75)[†]**
2-5 hours	35	22.9 (12.4-38.2)	**12.25 (3.34-44.89)[§]**	**7.26 (3.82-13.79)[§]**
≥6 hours	18	27.8 (11.7-52.7)	**15.90 (4.34-58.28)[§]**	**8.49 (4.89-14.74)[§]**
Unknown outdoor hours	216	4.2 (2.2-7.8)	1.80 (0.54-5.96)	1.09 (0.4-2.92)
Unknown outdoor access	119	15.1 (9.3-23.6)	**7.37 (1.58-34.41)[‡]**	1.70 (0.56-5.19)
Urban area[3]				
No	381	3.9 (2.4-6.5)	Ref.	
Yes	100	9 (4.5-17.3)	**2.41 (1.46-3.99)[§]**	
Unknown	189	12.7 (8.3-19)	3.55 (0.58-21.73)	
Woody area[3]				
No	468	4.7 (3.1-7.1)	Ref.	
Yes	13	15.4 (3.6-46.9)	3.69 (0.41-33)	
Unknown	189	12.7 (8.3-19)	2.95 (0.47-18.56)	
Rural areas[3]				
No	448	2.9 (1.7-5)	Ref.	Ref.
Yes	33	33.3 (19.2-51.2)	**16.73 (4.77-58.71)[§]**	**7.48 (2.4-23.35)[§]**
Unknown	189	12.7 (8.3-19)	4.87 (0.94-25.12)*	**5.39 (2.47-11.8)[§]**
Feeding raw meat				
No	314	6.7 (4.3-10.2)	Ref.	
Yes	170	2.4 (0.9-6.1)	**0.34 (0.13-0.88)[†]**	
Unknown	186	12.4 (8-18.7)	1.97 (0.37-10.53)	
Feeding raw fish				
No	400	5.5 (3.6-8.3)	Ref.	
Yes	81	2.5 (0.6-9.5)	0.43 (0.06-3.24)	

Factor	n	*Toxocara* prevalence %[1]	Univariable OR[1]	Multivariable OR[1,2]
Unknown	189	12.7 (8.3-19)	2.50 (0.39-15.85)	
Predation				
No	452	4.4 (2.8-6.9)	Ref.	
Yes	87	11.5 (6.2-20.3)	2.81 (0.81-9.66)*	
Unknown	131	13.7 (8.3-21.9)	3.44 (0.44-27.03)	
Sheltered in the last 6 months				
No	441	4.1 (2.5-6.6)	Ref.	
Yes	40	15 (7.5-27.6)	**4.15 (1.16-14.85)†**	
Unknown	189	12.7 (8.3-19)	3.42 (0.58-20.09)	
Preferential defaecation				
Indoor (litterbox)	370	2.4 (1.2-4.9)	Ref.	
Outdoor	29	27.6 (15.8-43.6)	**15.28 (9.61-24.3)§**	
Both indoor and outdoor	75	8 (3.7-16.6)	**3.49 (1.41-8.62)‡**	
Unknown	196	12.8 (8.4-18.9)	**5.86 (1.21-28.35)†**	
Frequency of litterbox cleaning				
No litterbox	39	23.1 (13.5-36.5)	Ref.	
≤1x/week	32	9.4 (3-25.8)	0.34 (0.12-1.03)*	
2x/week	69	2.9 (0.7-10.8)	**0.1 (0.06-0.17)§**	
≥3x/week	341	2.9 (1.5-5.6)	**0.1 (0.08-0.12)§**	
Unknown	189	12.7 (8.3-19)	0.48 (0.11-2.16)	
Diarrhoea				
No	431	4.9 (3.2-7.4)	Ref.	
Yes	50	6 (1.4-21.9)	1.25 (0.32-4.91)	
Unknown	189	12.7 (8.3-19)	2.84 (0.58-13.9)	
Discoloration in the stool				
No	470	4.9 (3.2-7.4)	Ref.	
Yes	11	9.1 (1.2-44.8)	1.94 (0.39-9.64)	
Unknown	189	12.7 (8.3-19)	2.83 (0.52-15.34)	
Gastrointestinal conditions				
No	226	9.3 (5.9-14.4)	Ref.	
Yes	53	5.7 (1.8-16.2)	0.59 (0.06-5.41)	
Unknown	391	6.1 (4.2-8.9)	0.64 (0.13-3.12)	
Cardiological and/or respiratory conditions				
No	246	8.9 (5.7-13.7)	Ref.	

Factor	n	*Toxocara* prevalence %[1]	Univariable OR[1]	Multivariable OR[1,2]
Yes	33	6.1 (1.8-18.7)	0.66 (0.05-9.19)	
Unknown	391	6.1 (4.2-8.9)	0.67 (0.13-3.37)	
Nephrological and/or metabolic conditions				
No	251	9.2 (6-13.8)	Ref.	
Yes	28	3.6 (0.5-21.7)	0.37 (0.02-6.99)	
Unknown	391	6.1 (4.2-8.9)	0.65 (0.12-3.53)	

** p≤0.10; † p<0.05; ‡ p≤0.01; § p≤0.001, OR = odds ratio*

[1] Adjusted for clustering at the levels of study cohort and household, [2]Adjusted for all variables whose ORs appear in this column, [3]The living environment 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, [4]One-sided, 97.5% confidence interval.

Owner's attitude towards deworming

Of the 335 cats tested for *Toxocara* and for which the deworming regimen was reported, 91 (27.2%) had never received an anthelminthic treatment according to the owner, 42 (12.5%) were treated at least once a year, 120 (35.8%) 2-3 times a year, and 82 (24.5%) ≥4 times a year. The frequency of treatment was not significantly associated with *Toxocara* positivity (Table 2). Of the 464 cats tested for *Toxocara* and for which the time since last deworming was known, 80 (17.2%) had received an anthelminthic treatment within 1 month before sampling, 160 (34.5 %) between 1 and 3 months, 95 (20.5 %) between 4 and 6 months, and 129 (27.8 %) more than 6 months before. The time of last deworming did not have a significant effect on the risk of being *Toxocara* positive (Table 2). There was no significant relation between the time the cat spends outdoors and the frequency of deworming.

Information on the main reasons for anthelmintic treatment was provided for 199 cats, corresponding to 81.6% of the cats for which a deworming regimen was implemented. The "cat's health" was the main reason to deworm for 160 cats (80.4%), followed by "public health" (21 cats, 10.6%), "because we must" (9 cats, 4.5%), and a combination of these (9 cats, 4.5%). There was no significant association between the main reason for deworming and the applied deworming frequency.

Discussion

Although infections with endoparasites are generally less studied in cats than in dogs, there are several reports on the prevalence of patent infections with *T. cati* in cats that indicate that cats are responsible for a considerable part of the environmental contamination with this zoonotic roundworm (Fisher 2003). In the Netherlands, the number of household cats exceeds the number of household dogs (HAS den Bosch and Utrecht University 2015) and, while there are no stray dogs, there is a large stray cat population (Neijenhuis and van Niekerk 2015). This, combined with the typical feline defaecation behaviour, leads to cats being responsible for a substantial contribution to the environmental contamination with *Toxocara* eggs and possibly the occurrence of toxocariasis in humans (Nijsse et al. 2015). Therefore, the public health relevance of *T. cati* should not be underestimated (Fisher 2003).

With an overall prevalence of 7.2%, cats in the Netherlands appear to be moderately infected with *T. cati* as compared to the mean European prevalence of 19.7% reported in 2014 (Beugnet et al. 2014). Our prevalence is lower than the one of 28.2% reported in 2004 among sheltered cats in the Netherlands (Robben et al. 2004), but it is comparable with prevalence rates in Germany (4.7-6.4%) (Barutzki and Schaper 2003; Barutzki and Schaper 2011) and the USA (7.5%) (Gates and Nolan 2009). However, it is much lower than the prevalence rates in areas that have comparable settings to the Netherlands, like Belgium, the northern part of Germany, and Denmark, with reported prevalences of 60% (Vanparijs et al. 1991), 27.1% (Becker et al. 2012) and 79% (Engbaek et al. 1984), respectively. The difficulty in comparing these prevalence rates derives from the different lifestyles within household cat populations and the concomitant differences in exposure to common risk factors. In Mexico City, the prevalence in apartment cats was only half of that found in other household cats, however, both these prevalences (20.7% and 42.5%, respectively) (Martinez-Barbabosa et al. 2003) were higher than that found in this study.

Studies focussing on risk factors for helminth infections in cats are scarce (Mircean et al. 2010; Beugnet et al. 2014). In our study, significant risk factors in the multivariable analysis were young age, living in rural areas, and roaming freely outdoors. Age is a known risk factor for ascarid infections of dogs and cats, though age resistance in household cats is probably less effective than in household dogs due to the predatory behaviour of cats (Overgaauw and van Knapen 2013). Age as a risk factor for cats was also described for cats in other studies (Mircean et al. 2010; Barutzki and Schaper 2011; Beugnet et al. 2014). The standard deworming advice for kittens states that they should be dewormed every two weeks from the age of three weeks until they are eight weeks of age, followed by monthly deworming up to six months of age

(ESCCAP September 2010). However, our data and those of other studies conclude that cats are at higher risk of developing patent infections up to one year of age.

Increasing time spent outdoors is a known risk factor for *Toxocara* infection in cats (Beugnet et al. 2014) and we observed an outdoor time-dependent relation with the risk of *Toxocara* infections, meaning that the more time a cat spends outside (unsupervised) the greater the risk of developing a patent infection. This may be related to the chance of ingesting infective eggs from the environment, but likely also to more time spent predating. However, predation itself did not prove to be a significant risk factor in the multivariable analysis. The reported predatory behaviour, however, is a reflection of what was observed by the owner/caregiver. When a cat is outside without supervision, the predatory behaviour can not always be witnessed with certainty, and unnoticed consumption of paratenic hosts might lead to patent infections. Living in a rural area is probably mirroring a higher chance for cats to encounter infective stages of *Toxocara*, either in the environment or in preys. Farm cats are usually free to roam in the surroundings and they are commonly a part of a farm's pest control plan by catching small rodents. The relation between living in rural areas and being at risk of developing patent *Toxocara* infections was also described by Mircean et al. (Mircean et al. 2010). Stray cats probably spend even more time unattended outside, exposed to the same factors, but are likely lacking any preventative veterinary care. Therefore, their contribution to environmental contamination with *Toxocara* eggs is assumed to be considerable (Fisher 2003; Morgan et al. 2013; Nijsse et al. 2015).

The lack of a significant association between the time since last deworming and patent *Toxocara* infection is surprising and needs to be further investigated. We also found that the advised deworming frequency of cats of at least four times a year was applied by 24.5% of the cat owners who reported their treatment regimen, meaning that 75.5% of those cat owners dewormed their animal less frequently. Most cat owners (80.4%) answering the question about the main reason for deworming their cats answered to do this because of their cats' health and only 10.6% answered that the primary reason was "public health". Both deworming frequency and incentive for deworming show that owners are not aware, and possibly misinformed, about why deworming is necessary. This remains a point of attention as reported before (Overgaauw and Boersema 1996; Overgaauw et al. 2009; Nijsse et al. 2014). A more custom-made deworming advice with attention for the risk factors of an individual cat could convince an owner to pay more attention to the deworming strategy of their cats.

Conclusively, our results show that about 7% of cats in the Netherlands shed *Toxocara* eggs. Besides young age and living in rural areas, we found that the more time a cat spends outdoors, the higher the risk for this cat to shed *Toxocara* eggs, indi-

cating that stray and free-roaming cats are more likely to contaminate their living environment with *Toxocara* eggs. The overall 7.2% prevalence in cats is higher than that observed in household dogs in the Netherlands (Nijsse et al. 2014). In conjunction with the fact that there are more cats than dogs, this implies that cats should receive more attention as a source of *Toxocara* eggs in the environment. Moreover, insufficient knowledge on the zoonotic aspects of *Toxocara* in combination with the low compliance to the advice of routinely deworming cats stresses the importance of educating cat owners about this parasitic infection of cats, the zoonotic risk and the rationale of following a (preferably risk-based) deworming regimen.

Acknowledgements

The veterinary students, performing their research internship at our division, Jolein Jansen, Lianne Koolwijk, Marjolein Koks, Sandra Vink, Manon Coenen, and Jorik Soontiëns are acknowledged for their substantial contribution to the laboratory work. The owners participating in this study and their cats are acknowledged for supplying faeces and answering the questionnaire.

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Chapter 5
Coprophagy in dogs interferes in the diagnosis of parasitic infections by faecal examination

Veterinary Parasitology (2014), 204: 304-309

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Toxocara V
lijnets/aquatint 2016

Abstract

Many dogs display coprophagic behaviour. Helminth eggs can passively pass the dog's digestive tract and this may result in a false positive diagnosis of infection with gastrointestinal helminth parasites. For a period of one year, faecal samples of dogs were examined monthly using the Centrifugal Sedimentation Flotation (CSF) technique with a sugar flotation solution (s.g. 1.27–1.30 g/cm^3). If a sample tested positive for canine helminth eggs, the owner was asked to submit another sample after preventing the dog from eating faeces for 3 days. If the second sample again tested positive for the same type of helminth egg, the dog was considered to have a patent infection. If the second sample tested negative, the first sample was considered a false positive due to coprophagy. The focus of this study was on dogs shedding *Toxocara* eggs. At the first examination, 246 samples (out of 308 samples testing positive for canine-specific helminth eggs) tested positive for *Toxocara* spp. Of these, 120 (49%) tested negative at the second examination.
Coprophagic behaviour was recognized by 261 of the 564 owners that answered the accompanying questionnaire. This concerned 391 dogs. Coproscopical examination also provided proof of coprophagy (e.g. oocysts of *Eimeria* spp. or non-dog typical helminth eggs) in dogs belonging to owners that did not report coprophagic behaviour in their dogs. Results indicate that coprophagy in dogs may result in an overestimation of the prevalence of patent helminth infections and that dogs may serve as a transport host for helminth eggs.

Keywords: Coprophagy, Coproscopical examination, Dogs, Roundworms, Nematodes, *Toxocara*

Introduction

Prevalence estimates of enteric helminth infections are usually based on cross-sectional studies in which finding helminth eggs in dog faeces at one point in time is considered as proof of infection. Some studies have suggested that coprophagy in dogs may be responsible for finding eggs of dog-typical (Sager et al., 2006; Ziadinov et al., 2008) as well as dog-atypical (Traub et al., 2002; Fahrion et al., 2011) helminth parasites in faecal samples in the absence of an actual infection. Generally speaking, coprophagy is likely to lead to an overestimation of the occurrence of patent helminth infections. The chance that eggs found during coproscopical examination originate from eating contaminated faeces, rather than from an actual infection, depends on the parasite species in question, as not all parasites produce eggs that pass through the gastrointestinal tract without being digested or at least morphologically affected. Ascarids for example produce robust eggs that have been shown to pass through the gastrointestinal tract seemingly unaffected (Traub et al., 2003; Deplazes et al., 2011). These eggs need to mature for a longer time in the environment to become infective (e.g. *Toxocara* spp.), and if ingested before reaching their infective stage they can passively pass through the gastrointestinal tract.

Coprophagy is a common behaviour among dogs. Dogs may consume their own faeces, faeces of other dogs and/or faeces of other species. Dogs consuming their own faeces are unlikely to affect prevalence estimates of patent infections. However, consuming faeces from other dogs may influence such estimates, especially as it may concern faeces from dogs at risk of harbouring patent parasitic infections. As the result of consuming faeces from other species, eggs of non-dog parasites that are hard to distinguish morphologically from eggs of dog parasites can also affect the results of coproscopical examinations.

It is unclear how frequently coprophagic behaviour occurs among dogs and to what extent coprophagy may influence prevalence estimates of cross-sectional studies on dog parasites based on single faecal examinations.

The aim of this study was to quantify the possible impact of coprophagy and associated passive passage of helminth eggs through a dog's gastrointestinal tract on the results of coproscopy based surveys. The focus was on *Toxocara* spp. as these worms produce robust ascarid eggs and *T. canis* is a parasite of zoonotic importance and is decisive for the deworming schedules in several countries. Other parasites, such as *Toxascaris leonina, Capillaria* spp., *Trichuris vulpis* and hookworms (producing strongyle type of eggs) were also considered.

Material and Methods

Study design
As part of a larger study on the (re)occurrence of and risk factors for gastrointestinal helminth infections in household dogs in the Netherlands, 901 dogs older than 6 months were included. Owners (n = 564) of these dogs subscribed voluntarily to the study and submitted faecal samples every month for coproscopical examination to the Faculty of Veterinary Medicine of Utrecht University between November 2012 and October 2013. Participants were instructed not to deworm their dogs during this project. Deworming of the dogs was only allowed after confirmation of a positive result by the project team, when a bitch was lactating or a dog was traveling abroad to a *D. immitis* endemic area. In the latter two cases the deworming product and moments of deworming were reported in the questionnaire. Data concerning the results of the coproscopical examination were communicated monthly to the owner.

If faecal samples scored positive for dog-specific helminth eggs by coproscopical examination, the owners were instructed to prevent their dogs from eating anything from the ground for at least three days based on reported gastrointestinal transit times in dogs (Boillat et al., 2010). Instructions involved emphasizing the reason to do this and included recommendations on how to keep dogs from eating anything from the ground (e.g. keeping the dog on a very tight leash). Following these three days another faecal sample was submitted. This sample was used to determine whether or not an infection could be confirmed. If the confirmation sample (CS) tested positive (positive confirmation sample, PCS) for the same types of parasite eggs found in the first sample, then the dog was considered patently infected. Otherwise the first sample was considered to have been passively contaminated by helminth eggs and the test result was considered negative (negative confirmation sample, NCS). Owners were instructed not to deworm their dogs unless a positive confirmation was reported to them.

After a PCS, an anthelmintic (Drontal Dog®) was sent to the owner for treatment of the dog and its efficacy was tested 14 days later, to make sure that the dog did not shed helminth eggs after treatment and could continue in the survey.

At the start of this study owners answered a questionnaire which, among others, contained questions concerning the owner's perception of coprophagic behaviour of their dogs, the living environment of the owners and purpose of the dogs. Data from this questionnaire were analysed even though owners did not actually submit any faecal sample from their dog(s) during the period of this study. Questionnaire data were used anonymously and the results of the coproscopical examination were communicated confidentially.

Faeces examination
For logistic reasons, two samples were pooled for first testing, but when they tested positive for dog-typical parasites they were retested separately to determine which of the samples contained the eggs. CS were not pooled.

The centrifugal sedimentation and flotation technique was used for coproscopical analysis of 3–5 grams of faeces using a sugar solution (s.g. 1.27–1.30 g/cm^3) as flotation medium. During the centrifugal flotation step, cover-slides were placed on top of the tubes. Slides were then microscopically checked systematically at 40x, 100x and 400x magnification. The major axes of eggs were measured using a micrometer in the ocular (Leitz periplan) of the microscope. If *Toxocara* spp. eggs were found, the sample was considered positive regardless of the sizes of the eggs. For other eggs, sizes as mentioned in the reference manual issued by the AAVP were used as guidelines for identifying eggs of dog typical parasites (Table1) (Zajac and Conboy, 2012).

Table 1. Sizes of helminth eggs used for determination by microscopic examination of dog faeces (Zajac and Conboy, 2012).

Species	**Size in µm**
Toxocara canis	85-90
Toxocara cati	65
Toxascaris leonina	75-80
Uncinaria stenocephala	71-92
Ancylostoma caninum	52-79
Trichuris vulpis	72-90
Capillaria aerophila	58-79
Capillaria boehmi	54-60

The number of eggs was scored semiquantitativily using “+++” for a slide that was filled with eggs, “++” for 1 egg in every field at 40x magnification, “+” for just several eggs in the total slide, “<+” for less than 6 in the total slide and “-“ for the absence of helminth eggs.

Oocysts of *Eimeria* spp. and helminth eggs from parasites that do not infect dogs as definitive hosts were used as a proof that the dog had eaten faeces of other animal species. When the same type of dog-specific helminth eggs were found in the CS, this was considered as a PCS, even if eggs or oocysts of typical non-dog parasites were present. Conversely, in the absence of the same type of dog-specific helminth eggs the sample was recorded as NCS.

Statistical testing
Questionnaire results and outcomes of coproscopical examination for helminth parasites are presented descriptively. Difference in median size of the major axis of *Toxocara* eggs between PCSs and NCSs was tested using the non-parametric Mann-Whitney U test. Differences in the frequencies of PCSs and NCSs among *Toxocara* egg size groups (≤84 µm, 85-90 µm, and ≥91 µm), according to the egg size reference values (Zajac and Conboy, 2012) and *Toxocara* egg count classes were tested using the Chi-square test. The same test was used to determine whether a NCS was significantly more likely to occur in dogs reported to show coprophagic behaviour. Statistical analysis was performed using the software SPSS and the significance level was set at $P<0.05$.

Results

Questionnaire results
The questionnaire was answered by 564 owners and concerned 901 dogs. A total of 561 owners (concerning 896 dogs) answered the question whether or not, in general, their dogs eat items from the ground (e.g. faeces, waste, grass, dead animals, etc.). Of these, 261 owners (47%) responded that their respective 391 dogs (44%) actually eat faeces (of unspecified origin). Most dogs (73%) were allowed to walk off leash, 7% of the dogs never walked off leash, and for the other 20% it was unknown. Of the owners that let their dogs walk off leash more than 50% of the time, 195 reported that their dogs were not coprophagic.

Most owners (55%) described their living environment as a residential area, 16% as rural, 7% as a wooded area and 22% as a combination of forementioned environments.

Table 2. Positive and negative confirmation samples found by microscopical coproscopy.

Positive samples (1st examination)		Confirmation samples (2nd examination)		
		Positive	Negative	Unknown*
Toxocara sp.	246	111 (45%)	120 (49%)	15 (6%)
Hookworms	60	22 (37%)	30 (50%)	8 (13%)
Trichuris sp.	19	7 (37%)	11 (58%)	1 (5%)
Capillaria sp.	18	0 (0%)	17 (94%)	1 (6%)

* *Dog owners did not provide a confirmation sample.*

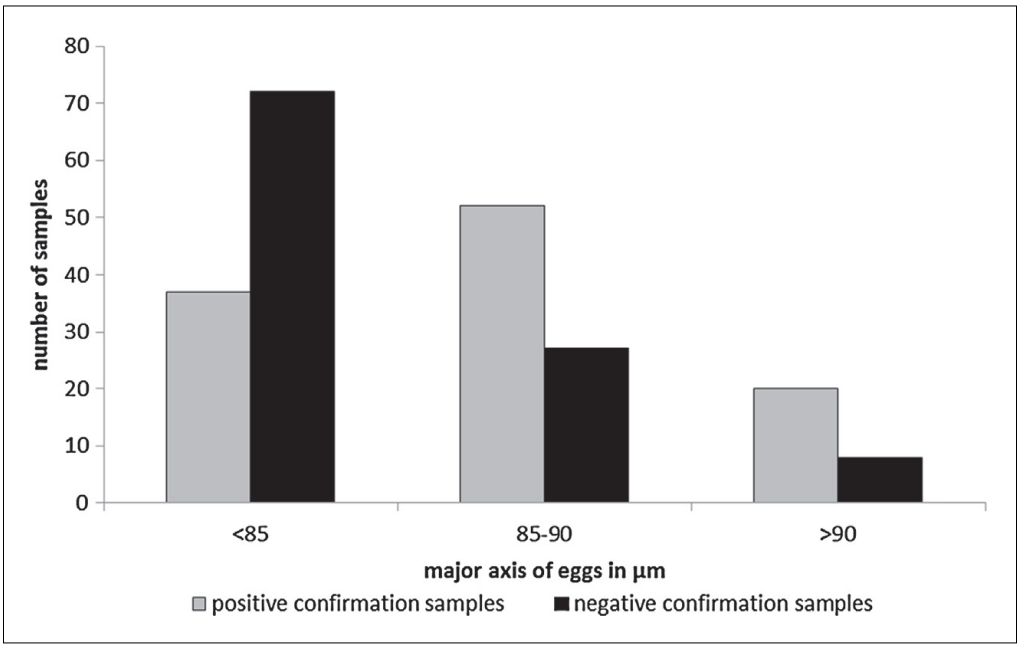

Fig. 1. Frequency distribution of Toxocara eggs by size group in samples that were either followed by a positive or a negative confirmation sample.

Parasite findings

Faecal samples of 219 dogs (belonging to 176 different owners) tested positive for dog-typical helminth eggs at least once. Table 2 shows the results of faecal examinations. At the first examination 313 samples tested positive for at least one dog-typical helminth parasite. Of these, 246 were positive for *Toxocara* spp., of which 120 (49%) were negative at the confirmation. Similar results were found for all other nematode species, except for *Capillaria* spp. for which none of the positive samples could be confirmed.

The measured sizes of the major axis of *Toxocara* eggs varied between 54 µm and 124 µm (median 84 µm). The median size of the major axis of *Toxocara* eggs in samples followed by a NCS (82 µm, range 54–96 µm) was significantly smaller ($P<0.001$) than those followed by a PCS (90 µm, range 68–124 µm). Small *Toxocara* eggs (<85 µm) were more often found in samples that were followed by a NCS than in samples followed by a PCS ($P<0.001$; Fig. 1). No significant difference was found between PCSs and NCSs with respect to the observed *Toxocara* egg count classes in the first sample.

Significantly more NCSs for *Toxocara* eggs (64.1%) were found in dogs with reported coprophagic behaviour ($P<0.05$). For other parasites no significant association was

found between NCSs and coprophagy. A NCS for *Toxocara* eggs was also significantly more likely to occur in dogs whose owners had reported seeing them ingesting unspecified materials from the ground (93.2%, P<0.01).

Typical non-dog parasite eggs and oocysts that were found during coproscopy were those of *Eimeria* spp., strongyle-type eggs that were too small or too large to be from canine hookworms, *Moniezia* spp., *Anoplocephala* spp. and *Heterakis / Ascaridia* spp.. Non-dog parasite eggs were present in 6% (n=18) of the primary samples that also tested positive for eggs of dog-typical parasites and in 8% (n= 26) of the confirmation samples of which 20 were a NCS. Thirty eight percent (17 out of 45) of the samples that contained eggs from typical non-dog parasites originated from dogs for which their owners did not report coprophagic behaviour.

No significant difference in the proportion of NCSs between the two different living environments (residential vs non-residential) was found.

Seven dogs tested positive for the same type of helminth eggs (*Toxocara* sp.) in two consecutive months and in both months the confirmation samples tested negative, although in 3 first samples and in 2 confirmation samples non-dog typical parasites/(oo)cysts were present.

Discussion and conclusions

Results show that prevalence estimates for patent helminth infections in dogs, that are allowed to walk outside, based on coproscopical examination need to be interpreted with caution. Forty-nine percent of all positive samples returned as NCSs, and also 49% of the samples containing *Toxocara* eggs returned as NCS.

Some authors (Robertson et al., 2000; Overgaauw et al., 2009; Macpherson, 2013) consider prevalence estimates based on the examination of single faecal samples to be a probable underestimation due to the possibility of intermittent shedding of helminth eggs. Intermittent shedding is described for infections with *Uncinaria stenocephala* (Rep and Bos, 1979) but, to our knowledge, intermittent shedding has never been described for *T. canis* infections in adult dogs. In some *T. canis* pre-infected and challenged adult silver foxes egg shedding showed a short but clear decrease followed by an increase in number of eggs per gram faeces (Saeed et al., 2005). However, in adult red foxes high egg counts were demonstrated (Richards and Lewis, 2001). In dogs this roundworm is known to be very productive, with an adult female reportedly being able to produce up to 200,000 eggs per day. It therefore appears unlikely that intermittent shedding was responsible for the high number of NCSs we found for this roundworm. Another possible explanation for the high number of

NCSs is that a dog experiences the final phase of a patent infection. However, the high number of observed NCSs and the fact that owners provided samples monthly for most of the dogs argues against this explanation. The average lifespan of adult *T. canis* worms has been reported to be four months (Parsons, 1987). Therefore an actual infection likely would have been noticed during the preceding month(s). The same may be true for a starting patent infection with a low egg count, as a faecal sample from a dog in this stage of infection should have tested positive in the following months. This was found in seven dogs of which the first sample tested positive for dog-typical helminth eggs in two consecutive months. However, in both months the confirmation samples tested negative, suggesting that a starting low patent infection was not likely. Moreover, the number of eggs that were shed in the first month by these dogs was not always low.

An alternative explanation for the high number of NCSs is coprophagy, which is supported by the significant association between NCSs and reported coprophagic behaviour. Coprophagy would imply an overestimation rather than an underestimation of the number of actual infections. This suggests that faeces containing helminth eggs is easily available for dogs. The source for faeces containing *Toxocara* eggs would be faeces from other dogs, cats, cattle or foxes. Faeces of cats and dogs is ubiquitously available. Although a case of *Toxocara vitulorum* in cattle has been reported recently in the Netherlands (Borgsteede et al., 2012), it is still considered not to be endemic and it is highly unlikely to be responsible for the NCSs. However, red foxes are common in the Netherlands and are known to shed eggs of *T. canis* (Borgsteede, 1984).

Based on reported egg sizes (Zajac and Conboy, 2012), faeces of wild rabbits may be the source of strongyle-type eggs passing the gastro-intestinal tract of a dog, as these fit the egg size of canine hookworms. *Trichuris* sp. eggs in dog faeces can be the result of eating faeces of mice and sheep and those of *Capillaria* sp. from eating bird faeces. To differentiate an infection from passive passage of eggs, the developmental stage of the eggs could have been of use. For example, when in fresh stool samples the eggs of *Toxocara* sp. show division of the zygote, a morula or other stages of development, it indicates that these eggs are only passing the gastrointestinal tract passively and cannot be the result of patent infections of the examined dogs. However, in this study, though it was observed, we did not systematically record developmental stages of eggs.

Apart from asking owners to restrain their dog from eating anything from the environment for three consecutive days and send in a new faecal sample, one could measure the size of the *Toxocara* eggs to differentiate between patent infection and contamination due to coprophagy. *T. cati* eggs should be smaller than *T. canis* eggs. Indeed, *Toxocara* eggs with a major axis smaller than 68 µm were found only

in samples followed by NCSs. According to the reference manual (Zajac and Conboy, 2012), these eggs better fit the size of *T. cati* eggs. This is also supported by an earlier morphological study by Uga et al. (2000), where the major axis of *T. canis* eggs varied from 71,6 – 91,2 µm and *T. cati* eggs from 63,7 – 88,1µm. However, the same authors also concluded that size is a poor determinant for discriminating between eggs of *T. canis* and *T. cati* because of the huge overlap in egg size. Nevertheless, the relatively large number of eggs in the NCS group that were smaller than the reference values for *T. canis* eggs suggests that the presence of *T. cati* eggs was responsible for a large part of the NCSs. Yet, larger eggs were also present in samples followed by NCSs, which can be explained by dogs eating faeces from other dogs or from foxes. Another way to discriminate eggs from non-dog parasites from morphologically similar eggs of dog parasites may be by using molecular techniques. However, molecular methods will not offer any solution for eggs found in faeces of dogs that have eaten faeces of other dogs or foxes (Ziadinov et al., 2008).

Besides the eggs of typical dog helminths, other helminth eggs and oocysts of parasites that do not infect dogs as a final host were found in the faeces samples. The presence of typical non-dog helminth eggs / oocysts occurred in samples with either a negative or a positive confirmation. Although finding these eggs or oocysts is a clear proof of coprophagy, it should not be regarded as a criterion for excluding the presence of a concurrent patent infection. Finding typical non-dog parasite eggs or oocysts in NCSs or PCSs implies that even though owners were asked to restrain their dogs from eating faeces for three days, they did not always comply fully with this request or missed their dog eating faeces stealthily. Consequently, this could also have resulted in false PCSs.

The number of NCSs was not significantly associated with the living environment of the dog. The lack of a significant association between living environment and PCS/NCS can be explained by the fact that the walking areas for dogs in the Netherlands can be completely different than their immediate living environment. Many owners living in a residential area walk their dog in a forest or rural area in weekends or holidays. Therefore, it is not surprising that a variable '(immediate) living environment' does not show significant differences in numbers of either PCS or NCS.

It is clear that dogs can serve as a mechanical vector of possibly viable eggs for a variety of helminth parasites. In some of the examined faecal samples stages of development of roundworm eggs were visible and larvae that were still alive were sometimes found in strongyle-type eggs. Deworming these coprophagic dogs will not prevent spreading of eggs due to mechanical transport. The apparent availability of faeces containing helminth eggs for consumption by dogs is indicative of the need of identifying the actual shedders in proximity of the examined animals, but also stresses the importance of cleaning up the faeces of a pet.

Structural coprophagic behaviour of their dogs was recognized by 46% of participating owners. However, given the results of the coproscopical examination, this percentage is likely to be conservative, as 38% percent of the positive samples that contained non-dog parasite eggs originated from dogs not eating faeces according to their owners. The number of dogs that frequently roam freely while their owners report the dogs not to be coprophagic also indicates that a dog-owner does not always know if their dog eats faeces.

In conclusion, this study shows that coprophagy is a widespread behaviour among household dogs.

Size, except for eggs smaller than 68 µm, that were only found in samples followed by a NCS does not necessarily provide information to distinguish between passive passage of eggs or patent infection by *Toxocara* spp.. The significant association between coprophagy and NCSs concerning *Toxocara* eggs indicates that cross-sectional prevalence estimates based on coproscopical examination of household dogs may suffer for up to 50% overestimation of patent infections.

Conflict of interest statement
The authors of this article wish to confirm that there are no known conflicts of interest associated with this publication. Bayer Animal Health was not involved in the study design nor in the interpretation of the results.

Acknowledgements
The authors thank Bayer Animal Health for their support of this study. The Veterinary Microbiological Diagnostic Centre of the Faculty of Veterinary Medicine of Utrecht University, Stephanie Rodenberg and students performing their research internship are thanked for their substantial contribution to the laboratory work. The owners participating in this study for supplying faeces and answering the questionnaire.

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Chapter 6
Environmental contamination with *Toxocara* eggs: a quantitative approach to estimate the relative contributions of dogs, cats and foxes, and to assess the efficacy of advised interventions in dogs

Parasites and Vectors (2015), 8: 397

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Illustratie: Wim Hendrikx
Toxocara VII
lijnets/aquatint 2016

Abstract

Environmental contamination with *Toxocara* eggs is considered the main source of human toxocariasis. The contribution of different groups of hosts to this contamination is largely unknown. Current deworming advices focus mainly on dogs. However, controversy exists about blind deworming regimens for >6-month-old dogs, as most of them do not actually shed *Toxocara* eggs. We aim to estimate the contribution of different non-juvenile hosts to the environmental *Toxocara* egg contamination and to assess the effects of different *Toxocara*-reducing interventions for dogs.
A stochastic model was developed to quantify the relative contribution to the environmental contamination with *Toxocara* eggs of household dogs, household cats, stray cats, and foxes, all older than six months in areas with varying urbanization degrees. The model was built upon an existing model developed by Morgan *et al*. (2013). We used both original and published data on host density, prevalence and intensity of infection, coprophagic behaviour, faeces disposal by owners, and cats' outdoor access. Scenario analyses were performed to assess the expected reduction in dogs' egg output according to different deworming regimens and faeces clean-up compliances. Estimates referred to the Netherlands, a country free of stray dogs.
Household dogs accounted for 39% of the overall egg output of >6-month-old hosts in the Netherlands, followed by stray cats (27%), household cats (19%), and foxes (15%). In urban areas, egg output was dominated by stray cats (81%). Intervention scenarios revealed that only with a high compliance (90%) to the four times a year deworming advice, dogs' contribution would drop from 39% to 28%. Alternatively, when 50% of owners would always remove their dogs' faeces, dogs' contribution would drop to 20%.
Among final hosts of *Toxocara* older than six months, dogs are the main contributors to the environmental egg contamination, though cats in total (i.e. both owned and stray) transcend this contribution. A higher than expected compliance to deworming advice is necessary to reduce dogs' egg output meaningfully. Actions focusing solely on household dogs and cats are unlikely to sufficiently reduce environmental contamination with eggs, as stray cats and foxes are also important contributors.

Keywords: *Toxocara* Eggs, Dogs, Cats, Foxes, Contribution, Contamination, Environment, Deworming, Clean-up

Background

Ocular and visceral larva migrans, as well as exacerbation of asthmatic allergies, are often associated with *Toxocara* spp. infection in humans [1][2][3]. This is supported by evidence from serological studies [2], although conclusive diagnosis can be very difficult [4] and seroconversion occurs often in people without recognized clinical symptoms [5].

Environmental contamination with *Toxocara* eggs is believed to be the main source of human infections, which are usually caused by accidental ingestion of infective eggs present in the environment. Of the different *Toxocara* species, *Toxocara canis* and *Toxocara cati* are considered to pose the highest zoonotic risk. Although there are incidental reports of *Toxocara vitulorum* [6], this species is not thought to be of significant epidemiological importance for human toxocariasis in the Netherlands. Therefore, in order to reduce the environmental contamination with *Toxocara* eggs, one should focus on the main egg shedders of *T. canis* and *T. cati*, i.e. dogs, cats, or foxes. Of these, dogs are probably the population of hosts in which *Toxocara* infections can be controlled the best by the owners, because, in contrast to cats, there is no notable population of stray dogs in the Netherlands.

The actual contribution of household dogs to the environmental contamination with *Toxocara* eggs is largely unknown, and so are the contributions of foxes and (either owned or un-owned) cats, which are commonly present in the Netherlands. A model quantifying the relative contributions of different final hosts to the environmental contamination with *Toxocara* eggs in the city of Bristol, UK [7], revealed that dogs, especially those in the age group of<12 weeks, were responsible for most of the total *Toxocara* egg output, even if it was assumed that 75% of the produced eggs did not reach the environment directly due to confinement of dogs at such a young age. Morgan *et al*. [7] further showed by simulation that the proportion of *T. canis* eggs reaching the environment is, not surprisingly, strongly dependent on the rates of removal of dog faeces by owners, but actual data about the compliance of dog owners to clean-up their dogs' faeces was not available and therefore could not be incorporated in the model. What also could not be considered in that model was the level of outdoor access of household cats, and the frequency of preferred use of the litterbox, or that foxes may have more or less access to some areas depending on their degree of urbanization. Accounting for the degree of access to different (outdoor) areas and removal of faeces is therefore likely to provide novel insights in the relative contributions of different hosts older than six months (hereafter referred to as non-juvenile hosts) to the environmental contamination by *Toxocara* eggs.

Currently, the European Scientific Counsel Companion Animal Parasites (ESCCAP) recommends to deworm adult dogs (>6 months of age) at least four times a year [8] to reduce the impact of patent infections on the environmental contamination with *Toxocara* eggs. However, this recommendation is not well supported by evidence and, as it is voluntary, it leaves ample room for dog owners to deworm their dogs (or not) in whatever frequency they like. As it cannot be expected that owners make these decisions based on adequate knowledge of the public health issues related to patent *Toxocara* infections [9], modelling the expected outcome of differing deworming frequencies might help determine the extent to which efforts should be put into convincing dog owners to comply with recommended treatment strategies. Because final hosts younger than six months of age are unlikely to have acquired age resistance against patent infections with *Toxocara* spp., they are believed to contribute by far the most to the overall *Toxocara* egg production [10][11][12][7]. Accordingly, the current deworming advice for these young animals, which is based on the prepatent periods of intra-uterine and lactogenic infection, as well as infection by ingesting embryonated eggs, should be propagated and enforced. This means that puppies are to be dewormed every two weeks up to the age of eight weeks, followed by monthly deworming up to the age of six months. The same applies to the advice of daily clean-up and disposal of their faeces by the owners. This advice is to be communicated to owners of puppies and kittens without reservation. There is, however, controversy about the necessity of the advocated deworming regimen for dogs older than six months, as the majority of household dogs (>90%) does not actually shed *Toxocara* eggs [13][14][15][9]. Additionally, for dogs older than six months, a mean prepatent period to serve as a guideline for deworming individual dogs cannot be as easily defined as in puppies. Puppies will not yet have developed an age resistance. Age resistance leads to mostly somatic instead of tracheal migration of larvae hatched from infective eggs. Therefore, when dogs have built up an age resistance, infection with embryonated eggs will not usually lead to a patent infection. Instead of migrating through the lungs, larvae cumulate in the somatic tissues which results in a prolonged and unpredictable prepatent period. For this reason, the present study focussed on animals older than six months, for which the propagated deworming advice is arguable.

Building upon the work of Morgan *et al*.[7], the main aim of this study was to develop a quantitative modelling approach to estimate stochastically the relative contributions of different non-juvenile host species to the environmental contamination with *Toxocara* eggs. Not only the host density, prevalence and intensity of infection, but also the degree of access to different (outdoor) areas and removal of faeces were taken into account. A comprehensive data set was then compiled using both published and original data to quantify the relative contributions to the overall *Toxocara*

egg output in the Netherlands of non-juvenile household dogs, foxes, owned and un-owned cats (hereafter referred to as stray cats), all older than six months. Another aim of this study was to assess the effects of implementing different deworming regimens and compliance to faeces clean-up policies for household dogs on the total environmental contamination with *Toxocara* eggs.

Methods

Modelling approach

Our modelling approach builds upon an existing model [7] to quantify the number of *Toxocara* eggs released into the environment by non-juvenile (≥6 month-old) final hosts (dogs, household cats, stray cats, and foxes) in the Netherlands. As there are virtually no stray dogs in the Netherlands [15], only the contribution of household dogs to the environmental contamination with *Toxocara* eggs was quantified. Conversely, both stray and household cats were considered.

The computational method used to estimate the overall daily egg output of non-juvenile dogs, household cats, stray cats and foxes (hereafter referred to interchangeably as hosts) in the Netherlands was the same for each of these hosts, with some adaptations depending on the data available and biological characteristics of the host in question (see Section 2.2). Since degree of urbanization and age are major determinants of host population size and frequency of egg shedding hosts [12][16][7][17][9], the degree of urbanization and the age structure were expected to have a strong effect on the estimates. Therefore, for all hosts, the daily egg output was estimated separately for young adults (6-12 months of age) and adults (>12 months of age), and for urban (>2500 addresses/km^2), intermediate (500-2500 addresses/km^2) and rural (<500 addresses/km^2) areas. The age categorization was based on a previous study [9] reporting a significantly higher risk of shedding *Toxocara* eggs in 6-12 month-old dogs compared to older age groups. The degree of urbanization, expressed in addresses/km^2 at the postal code area level, was based on the official categorization of the Dutch Central Bureau of Statistics used in other studies in the Netherlands, e.g. [18][19].

Description of the model

Let i denote the host, with i = 1 (dogs), 2 (household cats), 3 (stray cats), and 4 (foxes); let j denote the age group which individuals of host i belong to, with j = 1 (young adults) and 2 (adults); and let z denote the urbanization degree of the postal code area where individuals of host i and age group j live in, with z = 1 (urban areas), 2 (intermediate areas), and 3 (rural areas). The expected number of *Toxocara* eggs per km^2 released each day into the environment by host i of age group j living in area z,

denoted as E_{ijz}, is estimated as:

$$E_{ijz} \sim Poisson(\lambda_{ijz})$$
$$\lambda_{ijz} = D_{ijz} \times P_{ijz} \times F_i \times I_j$$

where D_{ijz} is the overall density (individuals/km^2) of host *i* and age group *j* living in area *z*; P_{ijz} is the true prevalence of patent *Toxocara* infections among individuals of host *i* and age group *j* living in area *z*; F_i is the average daily faecal output (grams of faeces per individual per day) of host *i* released into the environment; and I_{ij} is the average intensity of infection, expressed as eggs per gram of faeces (EPG), in host *i* and age group *j*. Full details on the estimation and data sources of these parameters are reported in Table 1. A sum of the egg outputs over age groups and areas, weighted by the size of the areas themselves (a_z, expressed in km^2), gives the overall daily egg output of host *i* in the Netherlands, denoted by:

$$E_i = \sum_j \sum_z E_{ijz} \times a_z$$

The model was based on a Monte Carlo simulation implemented in @Risk (Palisade Corp., USA) by setting 10000 iterations with the Latin hypercube sampling technique and a seed of one. Model convergence was monitored to check how statistics changed on the output distributions. Convergence testing was enabled every 100 iterations. Default convergence options were used, with a convergence tolerance of 3% and a confidence interval of 95%; all models showed optimal convergence.

Data sources and model parameterization

Dogs

The density of dogs by age group and urbanization degree (D_{1jz}) was obtained from a study on the pet population in the Netherlands in 2011 included in a report compiled by the University of Applied Sciences of Den Bosch and the Council of Animal Affairs in the Hague, the Netherlands, under the mandate of the Dutch Ministry of Economic Affairs, Agriculture and Innovation [20]. *Toxocara* egg prevalence in dog faeces by age group and urbanization degree (p_{1jz}) was obtained from a large study on the prevalence, risk factors and owners' attitude towards deworming for *Toxocara* based on 916 dogs of ≥6 months of age that was conducted in the Netherlands between July 2011 and August 2012 [9]. Dog owners voluntarily participated in this study and agreed on publication of the anonymised data. Such prevalence was adjusted for the likelihood for these dogs to display coprophagic behaviour, as this causes overestimation of the true prevalence due to the passive passage of helminth eggs through the dog's digestive tract following ingestion of "egg-contaminated" faeces [21]. Coprophagy-adjusted *Toxocara* egg prevalence in dog faeces was estimated as $P_{1jz} = p_{1jz} \times c_{1jz}$,

Table 1. Model parameters and sources, as used in the model. Parameter means are shown in Table 2.

Parameter	Description	Estimation	Source
Dogs			
D_{1jz}	Density of dogs of age group *j* in area *z*	Data	[20]
P_{1jz}	Prevalence of *Toxocara* patent infection in dogs of age group *j* in area *z*	$= p_{1jz} \times c_{1jz}$	See below
p_{1jz}	Coprological prevalence of *Toxocara* egg shedding dogs of age group *j* in area *z*	~Beta (a_{1jz}+1, b_{1jz}+1), where: $a_{1,1,1} = 2$, $b_{1,1,1} = 47$; $a_{1,2,1} = 5$, $b_{1,2,1} = 129$; $a_{1,1,2} = 10$, $b_{1,1,2} = 122$; $a_{1,2,2} = 12$, $b_{1,2,2} = 389$; $a_{1,1,3} = 6$, $b_{1,1,3} = 43$; $a_{1,2,3} = 7$, $b_{1,2,3} = 137$	[9]
c_{1jz}	Proportion of dogs of age group *j* in area *z* that do not display a coprophagic behaviour	~Beta (a_{1jz}+1, b_{1jz}+1), where: $a_{1,1,1} = 26$, $b_{1,1,1} = 22$; $a_{1,2,1} = 80$, $b_{1,2,1} = 54$; $a_{1,1,2} = 56$, $b_{1,1,2} = 75$; $a_{1,2,2} = 226$, $b_{1,2,2} = 175$; $a_{1,1,3} = 29$, $b_{1,1,3} = 18$; $a_{1,2,3} = 89$, $b_{1,2,3} = 55$	[9]
F_{1jz}	Average faecal output of a dog of age group *j* released daily into the environment of area *z*	$= f_1 \times s_{1jz}$	See below
f_1	Average faecal output of a dog	~Pert (21, 254, 1074)	[22][23][24][25][26][27][28][29][30][31][32][33]
s_{1jz}	Proportion of dog owners that do not comply to dog waste clean-up policies for dogs of age group *j* in area *z*	~Beta (a_{1jz}+1, b_{1jz}+1), where: $a_{1,1,1} = 20$, $b_{1,1,1} = 28$; $a_{1,2,1} = 80$, $b_{1,2,1} = 54$; $a_{1,1,2} = 87$, $b_{1,1,2} = 44$; $a_{1,2,2} = 277$, $b_{1,2,2} = 154$; $a_{1,1,3} = 27$, $b_{1,1,3} = 20$; $a_{1,2,3} = 106$, $b_{1,2,3} = 37$	[9]
I_{1j}	Infection intensity (EPG) for dogs of age group *j*	$I_{1,1}$ ~ Poisson (341.2); $I_{1,2}$ ~ Poisson (163.7)	[34]
Household cats			
D_{2jz}	Density of household cats of age group *j* in area *z*	Data	[20]
P_{2jz}	Prevalence of *Toxocara* patent infection in household cats of age group *j* in area *z*	~Beta (a_{2jz}+1, b_{2jz}+1), where: $a_{2,1,1} = 0$, $b_{2,1,1} = 2$; $a_{2,2,1} = 0$, $b_{2,2,1} = 18$; $a_{2,1,2} = 2$, $b_{2,1,2} = 15$; $a_{2,2,2} = 8$, $b_{2,2,2} = 52$; $a_{2,1,3} = 2$, $b_{2,1,3} = 1$; $a_{2,2,3} = 5$, $b_{2,2,3} = 12$	[Nijsse, unpublished data]

F_{2jz}	Average faecal output of a household cat of age group *j* released daily into the environment of area *z*	$= f_2 \times o_{2jz}$	See below
f_2	Average faecal output of a household cat	~Pert (10.2, 19.4, 52.4)	[35][36][37][38][39]
o_{2jz}	Proportion of household cats of group *j* in area *z* with outdoor access	~Beta (a_{2jz}+1, b_{2jz}+1), where: $a_{2,1,1} = 1$, $b_{2,1,1} = 1$; $a_{2,2,1} = 5$, $b_{2,2,1} = 13$; $a_{2,1,2} = 3$, $b_{2,1,2} = 13$; $a_{2,2,2} = 45$, $b_{2,2,2} = 13$; $a_{2,1,3} = 2$, $b_{2,1,3} = 1$; $a_{2,2,3} = 14$, $b_{2,2,3} = 3$	[Nijsse, unpublished data]
I_{2j}	Infection intensity (EPG) for household cats of age group *j*	$I_{2,1}$ ~ Poisson (372.8); $I_{2,2}$ ~ Poisson (81.7)	[40]
Stray cats			
D_{3jz}	Density of stray cats of age group *j* in area *z*	~Pert (135000,667500,1200000) × $D_{2jz}/(\sum_j \sum_i D_{2jz})$	Personal communication: preliminary estimate of feral cat project WUR Wageningen
P_{3j}	Prevalence of *Toxocara* patent infection in stray cats of age group *j*	~Beta (a_{3jz}+1, b_{3jz}+1), where: $a_{3,1,1} = 16$, $b_{3,1,1} = 12$; $a_{3,2,1} = 17$, $b_{3,2,1} = 8$; $a_{3,1,2} = 16$, $b_{3,1,2} = 12$; $a_{3,2,2} = 17$, $b_{3,2,2} = 8$; $a_{3,1,3} = 16$, $b_{3,1,3} = 12$; $a_{3,2,3} = 17$, $b_{3,2,3} = 8$	[11]
F_3	Average faecal output of a stray cat	~Pert (10.2, 19.4, 52.4)	[35][36][37][38][39]
I_{3j}	Infection intensity (EPG) for stray cats of age group *j*	$I_{3,1}$ ~ Poisson (372.8); $I_{3,2}$ ~ Poisson (81.7)	[40]
Foxes			
D_{4jz}	Density of foxes of age group *j* in area *z*	~Pert (0.5, 2.25, 4) × $d_{4jz}/(\sum_j \sum_i d_{4jz})$	[41]
d_{4jz}	Total number of foxes of age group *j* shot in area *z*	Data	[41]
P_{4jz}	Prevalence of *Toxocara* patent infection in foxes of age group *j* in area *z*	~Beta (a_{4jz}+1, b_{4jz}+1), where: $a_{4,1,1} = 1$, $b_{4,1,1} = 1$; $a_{4,2,1} = 1$, $b_{4,2,1} = 1$; $a_{4,1,2} = 18$, $b_{4,1,2} = 28$; $a_{4,2,2} = 9$, $b_{4,2,2} = 12$; $a_{4,1,3} = 57$, $b_{4,1,3} = 74$; $a_{4,2,3} = 19$, $b_{4,2,3} = 39$	[41]
F_4	Average faecal output of a fox	Log(F_4) ~ Normal (95, 18)	[42]
I_{4j}	Infection intensity (EPG) for foxes of age group *j*	$I_{4,1}$ ~ Poisson (157); $I_{4,2}$ ~ Poisson (366)	[12]

Description, estimation and data sources of the model parameters used to quantify the number of Toxocara *eggs released into the environment by non-juvenile (≥6 month-old) dogs, household cats, stray cats and foxes in the Netherlands.*

where p_{1jz} is the observed coprological prevalence of *Toxocara* eggs in dogs of age group *j* living in area *z*, and c_{1jz} is the corresponding age- and area-specific proportion of dogs that do not display a coprophagic behaviour as provided by Nijsse *et al*.[9]. Both p_{1jz} and c_{1jz} parameters were modelled as Beta distributions (see Table 1).

The average faecal output of a (Dutch) dog, denoted as f_1, was derived by calculating the pooled, sample size-weighted mean faecal output (expressed as grams of faeces per kilogram of dog's live body weight), over twelve different studies on dog food digestibility[22][23][24][25][26][27][28][29][30][31][32][33], weighted by the average bodyweight of a Dutch dog being 21.5 kg [20]. Minimum and maximum faecal outputs were derived proportionally by taking the Chihuahua and the Great Dane as reference breeds for the extremes of the dog faecal output range so that f_1 could be modelled as a Pert distribution (Table 1). Dog faecal output was adjusted for age- and area-specific likelihood for dog faeces to be cleaned-up by their owners as to estimate the amount of dog faeces that is actually released into the environment (F_1). This was estimated as $F_{1(jz)} = f_1 \times s_{1jz}$, where f_1 is the above mentioned average faecal output of a (Dutch) dog and s_{1jz} is the proportion of dog owners that does not comply to dog waste clean-up policies among those owning dogs of age group *j* living in area *z*. Parameter s_{1jz} was modelled as Beta distribution (Table 1) for which priors were obtained from Nijsse *et al*. [9].

Infection intensity (EPG) of *Toxocara* in dogs by age group (I_{1j}) was obtained from Sowemimo [34] and modelled as a Poisson distribution (Table 1). This parameter did not change over degrees of urbanization, but only over age groups, as it was assumed to be a parasite-related property in a given host, irrespective of the area that host lives in.

Household cats

The density of household cats by age group and urbanization degree (D_{2jz}) was obtained from the same source as dogs [20]. *Toxocara* prevalence in household cats by age group and urbanization degree (P_{2jz}) was obtained from a coprological study comprising126 owned cats in the Netherlands conducted at the Faculty of Veterinary Medicine of Utrecht University between October 2011 and February 2012 (Nijsse, unpublished data). Prevalence was modelled as Beta distribution (Table 1). All cat owners voluntarily participated in this study and agreed on publication of the anonymised data.

Similar to dogs, the average faecal output of a cat, denoted as f_2, was derived by calculating the pooled, sample size-weighted mean faecal output (grams of faeces per kilogram of cat's live body weight), over five different studies on cat food digest-

ibility [35][36][37][38][39]. Minimum and maximum faecal outputs were derived proportionally by taking the Singapura and the Maine Coon as reference breeds for the extremes of the cat faecal output range so that f_2 could be modelled as a Pert distribution (Table 1). Faecal output of household cats was adjusted for the age- and area-specific likelihood for household cat faeces to be actually released into the environment because these cats have access to outdoor areas. This was estimated as $F_{2(jz)} = f_2 \times o_{2jz}$, where f_2 is the above mentioned average faecal output of a cat and o_{2jz} is the proportion of household cats of age group *j* in area *z* having outdoor access. Parameter o_{2jz} was modelled as Beta distribution (Table 1) for which priors were obtained from the results of the above mentioned study (Nijsse, unpublished data).

Similar to dogs, EPG in household cats by age group (I_{2j}) was obtained from Sowemimo (2012)[40] and modelled as a Poisson distribution (Table 1), with no changes over degrees of urbanization.

Stray cats

There were no precise data on the density of stray cats by age group and urbanization degree in the Netherlands (D_{3jz}). At the time of writing, a survey to determine the number of stray cats in the Netherlands was ongoing at Wageningen University (http://www.wageningenur.nl/nl/project/Nederlandse-zwerfkatten-in-beeld.htm). They provided us with the most likely estimate of the stray cat population in the Netherlands based on their preliminary data. This estimate is between 135,000 and 1,200,000 stray cats. Using these priors, a Pert distribution was used to estimate the total stray cat population in the Netherlands, which was distributed over age groups and urbanization degrees based on the observed age structure and urban-to-rural gradient of household cats (Table 1). Inherent to this approach is the assumption that the stray cat population follows that of household cats in terms of both age composition and spatial distribution.

Toxocara prevalence in stray cats by age group (P_{3j}) was obtained from O'Lorcain [11] and modelled as Beta distribution (Table 1). Because of the lack of data, this parameter could not vary over degrees of urbanization, but only over age groups. The average faecal output of a stray cat was the same as that of household cats (Section 2.2.2), but it was not adjusted for outdoor access since by definition all stray cats live outside and all their faeces is released into the environment. EPG in stray cats by age group (I_{3j}) was the same as that of household cats (Table 1).

Foxes

There were no precise data on the density of foxes by age group and urbanization degree in the Netherlands (D_{4jz}). Franssen *et al*. [41] estimated an overall density of

0.5 to 4.0 foxes per km^2 in the Netherlands. Using these priors, a Pert distribution was used to estimate the average fox density in the Netherlands. This was then distributed over age groups and urbanization degrees based on the age structure and urban-to-rural gradient observed in a sample of 288 shot foxes submitted by hunters for routine inspection to the Dutch National Institute for Public Health and Environment between October 2010 and April 2012 [41] (Table 1). *Toxocara* prevalence in foxes by age group and urbanization degree (P_{4jz}) was also obtained from Franssen *et al*. [41], who examined the intestine of a subset of 262 foxes for the recovery of adult worms. Prevalence was modelled as a Beta distribution (Table 1). The mean and standard deviation of the faecal output of foxes were provided by Nissen *et al*. [42] so that the fox faecal output (F_4) could be modelled as a log normal distribution (Table 1). EPG in foxes by age group (I_{4j}) was obtained from Saeed *et al*. [12] and modelled as a Poisson distribution (Table 1), with no changes over degrees of urbanization.

Scenario analysis

Since dogs are the traditional target of control activities for *Toxocara* infection, different scenarios were simulated to quantify the impact of varying deworming regimens for dogs on the daily egg output of dogs in the Netherlands. These scenarios were run in parallel with those assessing the sole effect of removal of dog faeces. Sixteen scenarios were simulated in which four putatively advised deworming regimens (i.e. twice a year, four times a year, six times a year, and twelve times a year) were applied. For this simulation the use of short-acting deworming compounds is assumed at four different rates of compliance (i.e. 30%, 50%, 70% and 90%), with an average prepatent period of 30 days [43][44] and full efficacy of the deworming treatment. Since our model was based on real-world data, of which a subset was already used by Nijsse *et al*. [9], these scenarios were simulated on top of a background of observed deworming regimens and respective compliance rates present in the Dutch dog population (i.e. twice a year: 21.0% of dogs; four times a year: 17.5% of dogs; six and 12 times a year: unknown). Another four scenarios were simulated in which the observed compliance rates to dog waste clean-up policies (see Table 3) were increased by 20%, 50%, 70% and 90%.

Results

An estimated 84,100 (95%CI: 55,200-120,500) *Toxocara* eggs per km^2 per day are shed, on average, by non-juvenile hosts (>6 months) in the Netherlands. This corresponded to an average egg output of 1.46×10^6 (0.63×10^6-2.76×10^6) eggs per km^2 per day in urban areas, 109,500 (54,500-196,600) eggs per km^2 per day in intermediate areas, and 38,200 (21,200-61,700) eggs per km^2 per day in rural areas.

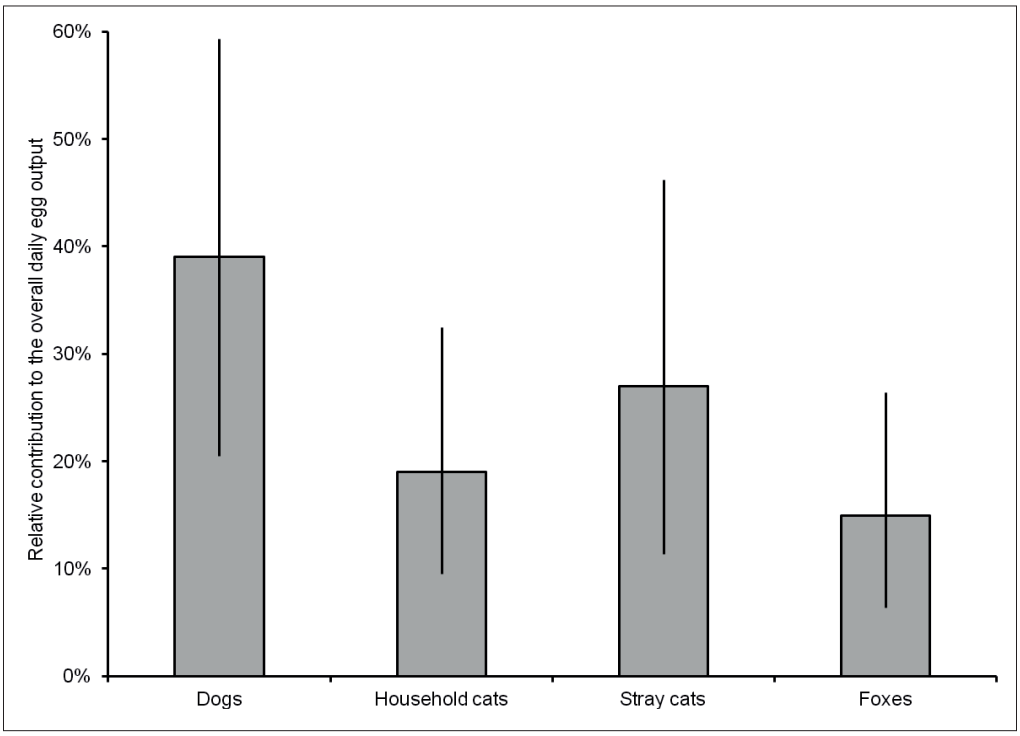

Figure 1. Estimated relative contributions (%) of dogs, household cats, stray cats, and foxes (all ≥6 month-old) to the environmental contamination with Toxocara *eggs in the whole of the Netherlands. Error bars represent 95% confidence intervals.*

Estimated host contributions to environmental egg contamination

Of the four putative non-juvenile hosts groups considered (dogs, household cats, stray cats, and foxes), dogs were estimated to be the most important contributor to the environmental contamination with *Toxocara* eggs (Figure 1). They accounted for 39.1% of the overall daily egg output of non-juvenile hosts in the Netherlands, followed by stray cats (27.0%), household cats (19.0%), and foxes (14.9%). This was in spite of the relatively low prevalence of patent *Toxocara* infections in dogs, but by virtue of their high population density and faecal output (Table 2), as well as low compliance of dog owners to dog waste clean-up policies (Table 3). However, when summing the contributions of household and stray cats together (46.0%), it appeared that non-juvenile cats as a whole are the primary contributor among the considered host groups. The relatively large population size and high prevalence of egg-shedding cats, either owned or stray (Table 2), along with a high proportion of household cats with outdoor access (Table 3), meant that non-juvenile cats were estimated to be the most important source of *Toxocara* eggs in the Netherlands, despite their relatively low faecal output and intensity of infection (Table 2).

Table 2. Estimated mean (with 95% confidence intervals) of the posterior distributions of model parameters.
Estimated mean and 95% confidence intervals of the posterior distribution of the host population den-

	Urban areas	
	Young adults	Adults
Population density (*D*), heads/km^2		
Dogs*	9	208.6
Household cats*	32.5	755.5
Stray cats	34.8 (15.1-54.4)	808.0 (352.7-1263.8)
Foxes	0.004 (0.002-0.006)	0.005 (0.002-0.007)
Prevalence (*P*), %		
Dogs§	3.2 (0.7-7.6)	2.6 (1.0-5.1)
Household cats	25.0 (0.8-70.8)	5.0 (0.1-17.6)
Stray cats***	56.7 (38.9-73.6)	66.7 (48.2-82.8)
Foxes**	50.0 (9.4-90.6)	50.0 (9.4-90.6)
Faecal output (*F*), g/day		
Dogs§§	147.7 (27.8-332.6)	209.6 (40.5-452.3)
Household cats§§§	11.7 (1.9-27.0)	7.0 (2.3-14.9)
Stray cats†	23.4 (12.1-39.5)	23.4 (12.1-39.5)
Foxes†	95.0 (64.6-134.9)	95.0 (64.6-134.9)
Infection intensity (*I*), eggs/g faeces		
Dogs††	341.2 (305-378)	163.7 (139-189)
Household cats††	372.8 (335-411)	81.7 (64-100)
Stray cats††	372.8 (335-441)	81.7 (64-100)
Foxes††	157.0 (133-182)	366.0 (329-404)

**Modelled deterministically as fixed single-point estimate, so no 95% confidence interval is calculated (see Table 1). **Derived from postmortem examinations of the intestine instead of copromicroscopy. ***Given the lack of detailed data, it did not change over urbanization degrees. §Adjusted for the rate of displayed coprophagic behaviour (see Table 3). §§Adjusted for the compliance of dog owners to faeces cleaning-up policies (see Table 3). §§§Adjusted for the rate of outdoor access (see Table 3). †Does*

sity, prevalence of patent Toxocara *infection, average daily faecal output released into the environment, and infection intensity for young adult (6-12 month-old) and adult (>12 month-old) dogs, household cats, stray cats and foxes in urban, intermediate and rural areas in the Netherlands.*

Intermediate areas		**Rural areas**	
Young adults	Adults	Young adults	Adults
3.4	79.7	0.4	8.7
5.7	131.8	0.5	12.5
0.3 (0.1-0.5)	6.9 (3.0-10.9)	0.01 (0.006-0.02)	0.3 (0.1-0.5)
0.3 (0.1-0.4)	0.3 (0.2-0.5)	0.7 (0.3-1.1)	0.9 (0.4-1.4)
3.5 (1.7-5.9)	1.8 (1.0-2.9)	8.4 (3.4-15.3)	3.4 (1.5-6.0)
15.8 (3.6-34.7)	14.52 (7.0-24.2)	60.0 (19.4-93.2)	31.6 (13.3-53.5)
56.7 (38.9-73.6)	66.7 (48.2-82.8)	56.7 (38.9-73.5)	66.7 (48.2-82.8)
39.6 (26.4-53.6)	43.5 (24.4-63.6)	43.6 (35.3-52.1)	33.3 (22.1-45.6)
232.9 (44.6-504.8)	225.9 (43.4-487.0)	201.1 (38.2-447.7)	259.6 (49.9-559.3)
5.2 (1.3-12.2)	17.9 (9.0-30.8)	14.0 (3.7-29.4)	18.5 (9.0-32.4)
23.4 (12.1-39.5)	23.4 (12.1-39.5)	23.4 (12.1-39.5)	23.4 (12.1-39.5)
95.0 (64.6-134.9)	95.0 (64.6-134.8)	95.0 (64.6-134.9)	95.0 (64.6-134.9)
341.2 (305-378)	163.7 (139-189)	341.2 (305-378)	163.7 (139-189)
372.8 (335-411)	81.7 (64-100)	372.8 (335-411)	81.7 (64-100)
372.8 (335-441)	81.7 (64-100)	372.8 (335-441)	81.7 (64-100)
157.0 (133-182)	366.0 (329-404)	157.0 (133-182)	366.0 (329-404)

not change over age groups and urbanization degrees since all stray cats and foxes release their faeces into the environment, so adjustments for outdoor access and compliance to faeces cleaning-up policies do not take place. ††Does not change over urbanization degrees, but only over age groups, as it was considered as a parasite-related property of a given host, irrespective of the urbanization degree where that host live.

Table 3. Estimated percentages of coprophagic behaviour, clean-up behavior of owners and outdoor access of household cats.

Area	Age group	Coprophagic dogs (c_1), %	percentage of dog owners that never/ rarely clean up feces (s_1), %	Household cats with outdoor access (o_2), %
Urban	Young adults	54.00 (40.23-67.46)	42.00 (28.81-55.78)	50.00 (9.41-90.56)
Urban	Adults	59.56 (51.22-67.62)	59.56 (51.22-67.63)	30.00 (12.57-51.20)
Intermediate	Young adults	42.86 (34.59-51-32)	66.17 (57.93-73.93)	22.22 (6.80-43.41)
Intermediate	Adults	56.33 (51.46-61.13)	64.20 (59.63-68.65)	76.67 (65.26-86.38)
Rural	Young adults	61.22 (47.34-74.23)	57.14 (43.21-70.51)	60.00 (19.39-93.24)
Rural	Adults	61.64 (53.64-69.34)	73.79 (66.36-80.60)	78.95 (58.56-93.59)

Estimated mean and 95% confidence interval of the posterior distribution of the rates of dogs displaying coprophagic behaviour, percentage of dog owners that never/rarely clean up feces, and outdoor access of household cats for young adults (6-12 month-old) and adults (>12 month-old) in urban, intermediate and rural areas in the Netherlands.

Host contributions to environmental egg contamination varied depending on the urbanization degree of the area in question (Figure 2). In urban areas, the overall daily egg output (0.97×10^9 eggs per day, corresponding to an average of 1.46×10^6 eggs per km^2 per day) was dominated by stray cats (80.7%), followed by dogs (15.0%), household cats (4.4%), and foxes (<0.01%). In intermediate areas, dogs were the main contributors (54.8%) to the overall daily egg output (1.48×10^9 eggs per day, corresponding to an average of 109,500 eggs per km^2 per day). In rural areas, the primary contributors to the overall daily egg output (1.05×10^9 eggs per day, corresponding to an average of 38,200 eggs per km^2 per day) were foxes (41.3%). These differences in contributions were the result of the relatively large population size of stray cats in urban areas and of foxes in rural areas, combined with a high density of dogs and household cats in intermediate areas (Table 2). Additionally, the presence of an urban-to-rural trend towards lower compliance of dog owners to dog waste clean-up policies and higher rates of outdoor access for household cats (Table 3) contributed to these differences. By contrast, foxes in urban areas and stray cats in rural areas were estimated to be few in number (Table 2), thus they appeared to contribute very little to the egg contamination in those areas.

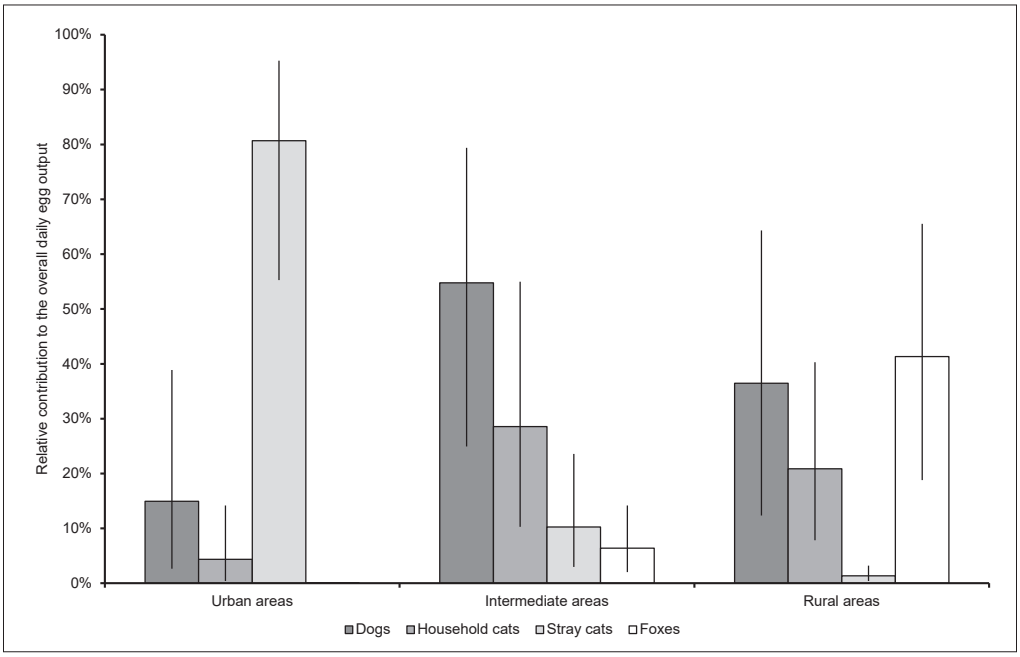

Figure 2. Estimated relative contributions (%) of dogs, household cats, stray cats, and foxes (all ≥6 month-old) to the environmental contamination with Toxocara *eggs in urban, intermediate and rural areas in the Netherlands. Error bars represent 95% confidence intervals.*

The daily egg output of each host was dominated by adults (>12 months of age) rather than young adults (6-12 months of age). This was in spite of the generally higher prevalence and intensity of patent *Toxocara* infections in younger animals, but driven by the much higher population size of the adult host populations (Table 2). Estimated contributions of adults relative to young adults of each host were 84.2% (95%CI: 63.3–95.7%) for dogs, 84.7% (67.1–95.5%) for household cats, 84.9% (72.2–93.3%) for stray cats, and 69.9% (56.6–80.9%) for foxes.

Effect of deworming regimen in dogs

The resulting estimated relative contribution to the environmental contamination of non-juvenile dogs in these different scenarios is shown in Table 4. By applying a deworming frequency of twice a year (i.e. once every six months), scenario analysis revealed that, compared to the current deworming frequencies applied by dog owners, the estimated percent reduction in the overall daily egg output by non-juvenile dogs in the Netherlands would vary from 3.3% (with a compliance rate of 30%), which amounts to a 37.8% overall contribution, to 13.8% (with a compliance rate of 90%), which amounts to an overall contribution of 33.7%. With a deworming frequency

Table 4. Estimated contribution of household dogs under different simulated deworming regimens and compliance rates.

	Deworming frequency (times/year)			
	2x	4x	6x	12x
Baseline compliance	21.0%	17.5%	Unknown	Unknown
Baseline contribution	39.1%	39.1%	39.1%	39.1%
Simulated compliance				
30%	37.8 (36.6 - 38.5)%	35.8 (32.9 - 37.6)%	33.7 (29.0 - 36.7)%	27.8 (18.0 - 34.4)%
50%	36.3 (33.9 - 37.9)%	33.0 (27.7 - 36.5)%	29.7 (21.5 - 35.0)%	19.9 (3.3 - 30.9)%
70%	35.0 (31.4 - 37.3)%	30.4 (22.8 - 35.5)%	25.7 (14.0 - 33.3)%	12.0 (0.0 - 27.5)%
90%	33.7 (29.0 - 36.7)%	27.7 (17.7 - 34.2)%	21.9 (6.7 - 31.7)%	4.1 (0.0 - 24.3)%

The estimated percent contribution (95% CI) of household dogs to the overall daily Toxocara *egg output under different simulated deworming regimens and compliance rates. Baseline compliance refers to the observed compliance rates according to Nijsse et al. [9].*

of four times a year (i.e. once every three months), the reduction was estimated to range from 8.5% (30% compliance) to 29.1% (90% compliance), while a deworming regimen of six times a year (i.e. once every two months) would lead to an estimated reduction ranging from 13.8 (30% compliance) to 44.1% (90% compliance). The estimated reduction of a twelve times a year deworming regimen (i.e. once every month) would vary from 28.8 (30% compliance) to 89.6% (90% compliance).

Effect of dog waste clean-up policies
By increasing the observed compliance rates of dog owners on top of the reported waste clean-up policies (Table 3) by 20%, 50%, 70% and 90%, the overall daily egg output of non-juvenile dogs in the Netherlands was estimated to be reduced to 32.2%, 20.1%, 12.0% and 4.0% respectively (Table 5).

Discussion

This study presents a quantitative approach for estimating the relative contributions of different host species, all older than six months of age, to the environmental contamination with *Toxocara* eggs, accounting for host density, prevalence and intensity of infection, as well as access to different areas and removal of faeces. Moreover, we

Table 5 - Estimated contribution of household dogs under different compliance rates of cleaning-up faeces by owners.

Compliance	Contribution to *Toxocara* egg output
20%	32.2% (36.4% - 26.7%)
50%	20.1% (31.2% - 3.1%)
70%	12.0% (26.1% - 0.0%)
90%	4.0% (24.3% - 0.0%)

Estimated percent contribution (95% CI) of household dogs to the overall daily Toxocara *egg output under different simulated compliance rates of cleaning-up dog faeces.*

assessed the effects of enforcing different deworming regimens and compliances to faeces clean-up policies for household dogs. Both published and original data were used, using the Netherlands as an example.

Even though raw meat is considered to be an important source of human *Toxocara* infections in other countries [45], infection through the ingestion of embryonated eggs from the environment is by far the most important route in the Netherlands and other Western European countries [4][15]. Infective *Toxocara* eggs can survive for several years in the environment; therefore, effective measures to reduce human

exposure to *Toxocara* should mainly aim at reducing the environmental contamination with eggs. Models like the one presented here are useful to attempt to quantify the sources of *Toxocara* eggs in a given locality as to prioritize control interventions and to assess the expected impact of such interventions. Morgan *et al*. [7] showed that the contributions of different hosts to the environmental contamination with *Toxocara* eggs can be quantified. Through appropriate modifications and use of additional data, our modelling framework can be extended to other regions with different urbanization degrees and different (compositions of) definitive host populations. Actual data on reported behaviors of non-juvenile dogs, cats and their owners concerning the applied deworming regimens and (compliances to) clean-up policies are included in the model. Of course leaving out the juvenile (<6-month-old) group of animals, which are unlikely to have developed age resistance, meant that the largest contributors to the environmental contamination by *Toxocara* eggs were not considered in this analysis and that emphasis was given to the larger adult host population, for which, unlike juvenile hosts, controversy exists about the need to deworm.

Our results revealed that cats contribute the most to the environmental contamination with *Toxocara* eggs by non-juvenile hosts in the Netherlands, although (household) dogs took over as the main contributors when household cats and stray cats were considered as two separate groups. This is in line with Morgan *et al.'s* model results [7]. However, when areas were stratified according to their degree of urbanization, host contributions appeared to differ greatly, with stray cats dominating in urban areas, dogs dominating in intermediate areas, and foxes in rural areas. The importance of cats as a putative source of *Toxocara* eggs has previously been emphasized and reported to be probably underrated [4]. Our results support the notion that controlling stray cat populations should be a priority in programmes aimed at reducing the contamination of the (urban) environment with *Toxocara* eggs. Defining the group of hosts responsible for the majority of *Toxocara* eggs shed in the environment is needed to assess the extent to which the advised *Toxocara*-control programmes may be expected to be successful in a given locality. For instance, based on our results, it seems that increasing the deworming frequency or the rate of faeces removal for non-juvenile dogs can be expected to reach the largest proportion of shedders, and also having the largest impact especially in the intermediate areas relative to urban or rural ones.

While the degree of urbanization mirrors the extent of suitable habitat for different definitive hosts, published data on the actual habitat preferences of foxes in the Netherlands are lacking. Our assumption about the distribution of the Dutch fox population over urbanization degrees was based on the urban-to-rural gradient observed in a convenience sample of shot foxes submitted by hunters for the screen-

ing for *Echinococcus multilocularis.* While it is clear that fox shooting is not usually practiced in urban areas to ensure the safety of the public, it is true that foxes have only sporadically been spotted in large Dutch cities (e.g. The Hague, Amsterdam, and Rotterdam)[46]. Therefore, most foxes appear to be dispersed over rural and intermediate areas relative to urban areas, although there may be some underestimation of the actual contribution of foxes in urban areas. For stray cats, instead, we assumed that their spatial distribution would resemble that of household cats. This meant that stray cats were found to be far more abundant in urban areas. Although it is conceivable that urban areas provide plenty of shelter and food to sustain large stray cat populations, it has been reported that stray cat dispersal might differ over seasons and different types of habitats [47][48]. This would imply that our contribution to environmental contamination with *Toxocara* eggs of non-juvenile stray cats in urban areas might be overestimated due to insufficient insights in the spatio-temporal pattern of this cat population. Moreover, the population of stray cats in the Netherlands is actually composed of both feral (sylvatic) cats and, previously owned, abandoned stray cats which might prefer different habitats. Because key characteristics of landscape use of stray cats in the Netherlands are lacking and information about the actual dispersal of the stray cat population is scarce, outcomes of the model could not be differentiated further. However, in this study, the tendency of cats to dwell in areas with high availability of food and shelter has been decisive to assume the preference for urban areas. Future studies should focus on differentiating the contributions of these feline subpopulations, including their egg shedding patterns, habitat preferences, population structure, and possible contacts with humans.

Apart from the need to acquire more specific information about each host population, several other limitations in the model can be identified. As information in literature about the mean reproductive worm burden in adult hosts is lacking, our model made use of known EPG-values as a measure of the intensity of infection [12][34][40]. Modelling the number of egg-producing worms present in the intestines and their fecundity in animals older than six months would have probably been a more biologically sound approach. We speculate that this would have probably led to a reduction in the maximum number of eggs shed by large-sized dogs as the number of adult worms per host is not expected to be linearly correlated with its bodyweight, but rather with the dose of infective eggs/larvae ingested. Given the hosts we considered here, this assumption will have the largest effect on the modelled canine egg output, as the different breeds of dogs show the largest variation in bodyweight.

As mentioned earlier, we focussed on dogs older than six months because younger dogs are known to be *Toxocara* egg shedders of paramount importance [49][10][7]. Consensus exists that in this young age group, the propagated deworming regimen

[8] and proper disposal of faeces must be enforced in any case. Conversely, the rationale of recommendations to control *Toxocara* infections in adult animals is much more arguable. If <6-month-old animals were included in the model, their contribution would have probably surpassed that of non-juvenile hosts, while the deworming advice for this age group would in fact remain the same.

The scenario analysis revealed that only in the case of a high compliance rate to a high deworming frequency (i.e. ≥50% of owners deworming their dogs twelve times a year), the contribution of non-juvenile household dogs could be expected to be halved. It is unclear what rate of voluntary compliance to a given deworming regimen would be feasible to reach in the Netherlands or in any other country. Several studies in the Netherlands have reported a compliance of circa 40% for deworming at least twice a year, but this was observed after conducting a campaign propagating deworming via the media or by asking clients visiting a veterinary clinic [50][15]. Customized advice for dogs frequently shedding eggs or dogs at high risk of shedding might be more efficient in reducing the contribution of non-juvenile household dogs to the environmental contamination [9]. Blind treatments at different frequencies do not appear to be as successful as may be expected [13][51][9]. Considering that only about 5% of non-juvenile household dogs actually are shedding *Toxocara* eggs at a given moment in time [14][15][52][9], the question is legitimate whether it is worthwhile to invest in a policy of frequent blind treatments. The same can be said for the clean-up of dog faeces, though enforcement of mandatory removal of dog faeces is perhaps more realistic, and our model showed that this would lead to results comparable to those that can be obtained with frequent deworming. Additional benefits (esthetical and hygienic) of the removal of dog faeces from the environment can play a decisive role in defining the priority of interventions. Both deworming and faeces removal were simulated separately, but the outcome of simulations assessing interaction effects between the different policies and compliances might differ from those assessing these effects independently of one another. It is therefore recommended that future studies assess these interactions and collect more information about incentives for dog owners to comply to one and/or to another policy. In addition, it is worth mentioning that we assumed an overall efficacy of 100% for the deworming intervention, but this might not always be the case under field circumstances. Together, these results would make the (mandatory) clean-up of faeces a more pursuable *Toxocara*-control option than deworming *per se*.

Finally, because of the different defecation behaviors of household dogs, household cats, stray cats, and foxes, and the likely differences in the longevity of *Toxocara* eggs in the environment associated with these behaviors, our results might not entirely reflect the origin of the eggs actually present in the environment. Our model, there-

fore, was only able to predict the relative contributions of different hosts to the total number of eggs released into the environment, but not to the chance of their recovery some time afterwards.

In conclusion, a quantitative model is presented with which the relative contributions of different host species to the environmental contamination with *Toxocara* eggs can be estimated. This model expands on the previously published model of Morgan *et al*. [7]. Filling in gaps in current knowledge will improve the quality of data gathered to inform the model, providing more precise evidence about the most promising targets and strategies to reduce the environmental contamination with *Toxocara* eggs.

Competing interests
The authors declare that they have no competing interests.

Consent to publish
All the dog and cat owners voluntarily participated in this study and agreed on publication of the anonymised data.

Acknowledgements
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 faecal samples of their pets and answering the questionnaire. Dr. Ibon Tamayo-Uria is acknowledged for his support in spatial data translation. The authors also like to thank Paul Overgaauw and Market Response for sharing the data of the report 'Feiten en Cijfers". Francesca Neijenhuis is acknowledged for sharing the preliminary estimation of the size of the Dutch stray cat population.

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Chapter 7
Recurrent patent infections with *Toxocara canis* in household dogs older than six months: a prospective study

Parasites and Vectors, In press

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Illustratie: Wim Hendrikx
Toxocara VIIb
lijnets/aquatint 2016

Abstract

To reduce environmental contamination with *Toxocara canis* eggs, the current general advice is to deworm all dogs older than six months on average four times a year. However, only a small proportion of non-juvenile household dogs actually shed *T. canis* eggs, and some dogs shed eggs more frequently than others. The identification of these frequent shedders and the associated risk factors is an important cornerstone for constructing evidence-based deworming regimens. The purpose of this study is to identify risk factors associated with recurrence of periods of shedding *Toxocara* eggs in a cohort of household dogs older than six months.

We performed a prospective study (July 2011-October 2014) on shedding *Toxocara* eggs in a cohort of 938 household dogs older than six months from all over the Netherlands. The median follow-up time was 14 months. Monthly, owners sent faecal samples of their dogs for *Toxocara* testing and completed a questionnaire. Dogs were dewormed only after diagnosis of a patent infection (PI). Survival analysis was used to assess factors influencing the time to first diagnosed PIs and the time to recurrent PIs.

The overall prevalence of PIs was 4.5%, resulting in an estimated average incidence of 0.54 PIs per dog/year. No PI was diagnosed in 67.9% of the dogs, 17.5% of the dogs went through only 1 PI and 14.6% had >1 PI. Prevalence of PIs always peaked during wintertime. Increased hazards for first diagnosed PIs were associated with coprophagy, geophagy, walking off-leash for ≥80% of walking time, reported worms in the faeces, feeding a commercial diet, and suffering from urologic or respiratory conditions. Median time to reinfection was 9 months. Factors associated with increased hazards for recurrent PIs were taking corticosteroids, changing dog's main purpose, and proxies for veterinary care-seeking behaviours.

We concluded that targeted anthelmintic treatments in household dogs may be feasible as PIs tend to (re)occur in specific periods and in groups of dogs at high risk. Moreover, recurrent PIs appear to be influenced more by factors related to impaired immunity than environmental exposure to *Toxocara* eggs.

Keywords: Deworming, Dogs, Recurrent patent infections, *Toxocara canis*, Longitudinal study

Introduction

Toxocara canis is a worldwide-distributed parasitic roundworm of canids with recognized zoonotic potential [1]-[4]. In patent infections, adult *T. canis* worms live in the intestine of dogs and other canids, laying eggs that pass into the faeces and contaminate the environment [5]. Within these eggs, a third stage larva develops, after which the eggs are infective. This embryonation process usually takes several weeks [6],[7]. Like other paratenic hosts, humans can become infected by ingesting embryonated eggs or larvae in raw or undercooked meat.

In young dogs (≤6 months of age), the ingestion of infective *T. canis* eggs is most likely to lead to hepato-tracheal migration of the larvae followed by a patent infection. Conversely, the ingestion of infective eggs by older dogs (>6 months of age) is less likely to lead to patent infections, as dogs develop immunity against the tracheal migration of the larvae [8],[9], resulting in so-called somatic migration [10]. This migration route leads to larvae residing somewhere in a dog's body where they can survive for long periods, but it does not lead to a patent infection. Therefore, most dogs older than six months do not actively contribute to the environmental contamination with *T. canis* eggs. Yet, some dogs older than six months do occasionally develop patent *T. canis* infections [11]. This is likely due to insufficient levels of built-up immunity or to temporary changes in immunity, e.g. because of endocrinologic perturbations, immune disorders, or stress. Also the uptake of low numbers of infective eggs [9],[11],[12] or the infection with larvae (rather than infective eggs) by consumption of raw meat and offal from infected paratenic hosts can lead to patent infections in adult dogs due to the evasion or avoidance of acquired immunity on lung level [10].

The fact that a few dogs older than six months do shed *T. canis* eggs [13]-[16], posing a risk for human infection, is used to justify the current "preventive" 3-to-4-times-a-year blind deworming advice for household dogs in this age category [17]. However, it has not yet been proved that such a treatment strategy is effective in reducing the contamination of the environment [16],[18], whilst it does lead to numerous treatments administered in absence of an actual patent infection to be treated. Therefore, monthly or three-monthly faecal examinations are also recommended as a feasible alternative to "preventive blind treatment" [17]. To implement evidence-based treatment strategies for dogs, it is crucial to identify dogs that are prone to develop patent *T. canis* infections [13],[19]-[21]. In young dogs or in dogs infected with larvae instead of eggs, a defined prepatent period can be used for preventive treatment. For most other dogs, however, a suitable interval is less obvious because the acquired immunity will prevent the development of patent infections or prolong the prepatent period to variable extents following ingestion of infective eggs. Cross-

sectional studies in North-European countries show that, at any given point in time, about 5% of household dogs shed *T. canis* eggs in their feces [14]-[16],[22],[23]. However, such studies usually fail to show to what extent and at what interval dogs older than six months experience recurrent *T. canis* infections. Adult dogs that are frequent egg shedders are more suited targets for regular treatments. To address the occurrence of recurrent *T. canis* infections in non-juvenile dogs, we performed a longitudinal study comprising a large cohort of household dogs older than six months in the Netherlands. The aim of this study was to determine the frequency of, and factors associated with, recurrent patent *T. canis* infections in these dogs.

Materials and Methods

Study design and dog population

Each month for a maximum period of 40 months (July 2011 to October 2014), dog owners in the Netherlands were asked to submit a faecal sample of their dog(s) to be examined for the presence of helminth eggs (see below) at the faculty of Veterinary Medicine of Utrecht University. Along with each submitted sample, owners were asked to complete a web-based questionnaire to collect relevant epidemiological information (see below). Dog owners were enrolled via advertising the opportunity of enrolment in the study across pet shops, veterinary clinics, pet-themed websites and dog breed societies in the Netherlands. Additionally, flyers were handed out at some dog walking areas. Recruitment of dogs from already participating owners was allowed during the entire study period. To be enrolled in the study, dogs had to be at least six months of age and, for logistic reasons, each owner was allowed to enrol a maximum of four dogs. Laboratory results were sent monthly by e-mail to the participating dog owners. Once enrolled in the study, dogs were not allowed to be dewormed unless a positive laboratory result was obtained, the dogs were traveling to *Dirofilaria immitis*-endemic areas, or they were lactating and performing litter care. In case of a positive laboratory result, the owners were asked to prevent their dog from eating anything from the ground for at least 3 days and send in a new sample. This step was included to rule out positive samples due to coprophagy as much as possible [24],[25]. If this confirmation sample tested positive also, it was considered a patent infection. After a positive confirmation sample a short-acting anthelminthic product (containing febantel, pyrantel and praziquantel) was provided. If a parasitic infection (e.g. *Cysto-isospora* spp.) was diagnosed that could not, either legally or due to suboptimal efficacy, be cured with this anthelminthic, owners were advised to confer with their veterinarian.

Owners participated in this study knowing that the acquired data would be used for a scientific publication.

Collection of epidemiological data
Epidemiological data were collected via a self-administered questionnaire that could be answered online. We differentiated between the starting questionnaire (completed at submission of the first faecal sample) and the follow-up questionnaires, which were completed at submission of each subsequent sample. The starting questionnaire contained questions about the dog's age, sex, breed, function, reproductive status, living conditions, diet, time roaming freely, predatory and coprophagic behavior, health status, medication use, and deworming history. The follow-up questionnaires were meant to monitor any change in living conditions, lifestyle (e.g. diet, function, etc.) or health of the dogs relative to the preceding questionnaire. Owners were specifically asked to report whether and when their dogs had been dewormed for reasons other than those provided above. A copy of the questionnaires is available as supplementary data. Information on socio-economic status (SES, a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area) and urbanization degree (>2000, 1500–2000, 1000–1500, 500–1000, and <500 addresses/km2) was obtained at the postal code level from Statistics Netherlands (http://www.cbs.nl/en-GB/menu/home/default.htm).

Coproscopical examination
Samples were submitted individually from each dog using a collection box at the faculty of Veterinary Medicine of Utrecht University (for people living or working close by) or submitted to the laboratory by regular mail, using study-provided materials and instructions. Each sample was identified by a unique code, which was linked to the questionnaire. The centrifugal sedimentation and flotation technique was used for coproscopical analysis [16],[25],[26]. For each sample, at least three grams of faeces was used and a sugar solution (s.g. 1.27–1.30 g/cm^3) was used as flotation medium. This method has a theoretical detection limit of detecting 1.6 eggs per gram. Slides were microscopically examined at 40×, 100× and 400× magnification. *T. canis* eggs were measured and morphologically identified, using the AAVP reference guide for diagnosing parasitism in animals [27]. For logistic reasons, two samples (three grams each) were pooled in the laboratory for first testing, with a theoretical detection limit of 3.2 eggs per gram for each individual dog in the pooled sample. If this pooled sample tested positive for dog-typical parasites, the samples were re-tested separately to determine which sample contained the eggs.

Data analysis
Survival analysis was used to assess factors influencing the time to the "first" diagnosed event of *Toxocara* egg shedding (first patent infection = FPI) and the time to recurrence of a patent infection (recurrent patent infection = RPI) in our dog population. This was done using Cox proportional hazards models, which assessed the risk

of patent *Toxocara* (re)infection longitudinally as a function of the factors measured at each sampling event. For the time to FPI, dogs entered the cohort at the submission of the first sample and were censored at their first diagnosed infection. Observation time for the time to FPI was then calculated as the time from the submission of the first sample (i.e. enrolment in the study) to that of the FPI or the end of the follow-up period (i.e. end of study or dropout from study). For the time to RPI, entry into the cohort began with the FPI and dogs were not censored after each subsequent reinfection. A conditional risk set model [28], in which the analysis is stratified by event (i.e. infection) order, was used for the analysis of the time to RPI. The assumption is that the conditional risk at time t for event k derives from all subjects under observation at time t that have had event $k - 1$. The method is widely used for analysis of recurrent events in the biomedical literature [29]. Observation time for the time to RPI was then defined as the gap time between subsequent infections (i.e. time to each event is measured from the previous event), or from the FPI to the end of the follow-up period (end of study or dropout from study) if the dogs did not have a RPI. Associations were expressed as hazard ratios (HRs) with 95% confidence interval (95%CI).

Preliminary analyses included log-rank tests for equality of survivor functions and Kaplan-Meier curves to assess graphically the assumption of proportionality for Cox proportional hazards for each independent variable. Variables satisfying these conditions were selected for inclusion in a multivariable Cox proportional hazard regression model. A backward stepwise selection procedure was then applied, with variables showing a $p \leq 0.05$ for the association with the outcome variable being retained in the model. The effect of removing variables on the associations of the other covariates was also monitored. A change of ≥10% in the coefficients was considered as a sign of confounding and the variable in question was retained in the model regardless of significance. The variables dog's age (6-12 months, 1-7 years, >7 years), sex, season (winter, December-February; autumn, September-November; spring, March-May; summer, June-August), time since last deworming (continuous variable expressed in months), and reported coprophagic behaviour were always controlled for in the models. The tested variables are intrinsic to the questions in the questionnaire, which is available as supplementary data. The SES was included as test variable, obtained at postcode level. Biologically plausible interactions between covariates were also assessed and the final model was expanded to include significant interaction terms, if any. Besides the repeated measurements made on the same dogs over time (multiple-record-per-subject analysis), we accounted for clustering (or non-independence) of dogs living in the same household (i.e. having the same owner) by incorporating cluster-robust variance estimators. Statistical analysis was performed using STATA 13 (StataCorp LP, College Station, USA).

Results

Descriptive statistics
In total, 938 dogs belonging to 570 owners were enrolled in the study. The cohort was followed for a total of 12,968 dog-months. Figure 1 shows the distribution of dogs over the number of months of follow-up. The median follow-up time per dog was 14 months (interquartile range [IQR] 5-22 months). The median age of the dogs at enrolment was 4 years (IQR 2-7 years). The study population consisted of 406 (43.3%) males and 532 (56.7%) females (male/female ratio = 0.76).

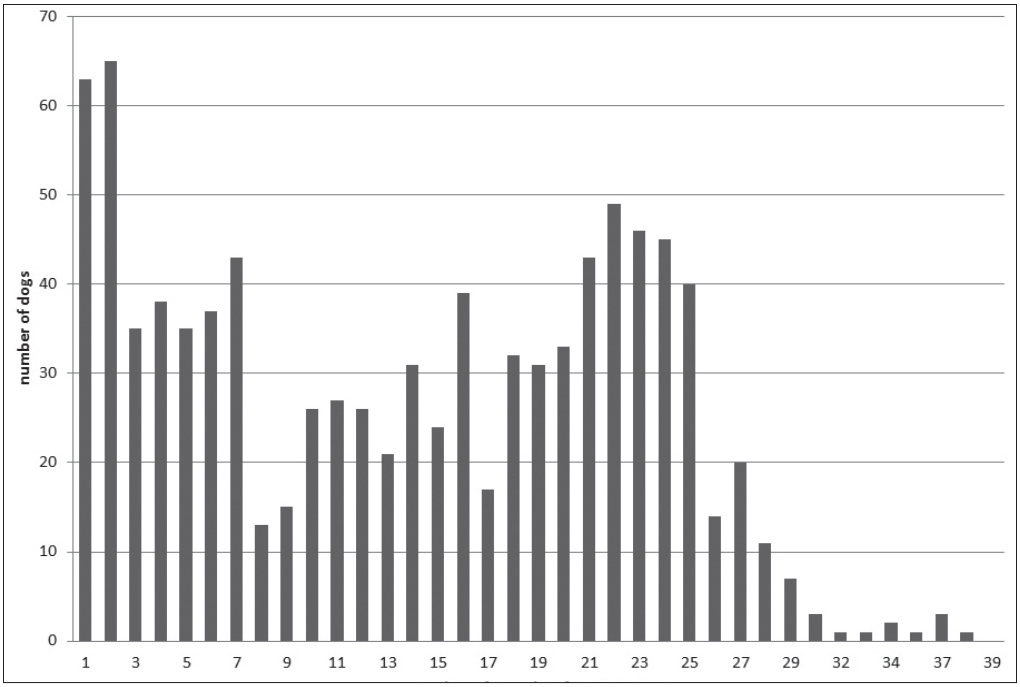

Fig. 1. The distribution of duration of participation in months with the corresponding number of dogs.

Of 12,968 stool samples tested, 585 were positive for *Toxocara* eggs, resulting in an overall proportion of 4.5% (95%CI 4.0-5.1%) positive samples. Table 1 shows the number of dogs and corresponding number of samples stratified by their number of positive test months diagnosed during the study period. In total, 301 (32.1%) dogs had at least one *Toxocara* infection, whereas the remaining 637 dogs (67.9%) never tested positive. The incidence rate was estimated at 0.54 patent *Toxocara* infections (95% CI 0.48-0.61) on average per dog/year. Anthelmintic treatment was given in 84 occasions for reasons unrelated to the study (e.g. foreign travel), in these cases dogs were allowed to continue their enrollment in the project.

Table 1. Dogs and samples stratified by number of Toxocara *eggs positive test months.*

Number of patent infections	Number of dogs	Samples			
		n	*Toxocara* negative	*Toxocara* positive	Mean number of samples per dog
0	637 (67.9%)	7706	7706	0	12
1	164 (17.5%)	2761	2597	164	17
2	66 (7.0%)	1188	1056	132	18
3	33 (3.5%)	566	467	99	17
4	18 (1.9%)	347	275	72	19
5	9 (1.0%)	174	129	45	19
6	8 (0.9%)	164	116	48	21
8	2 (0.2%)	38	22	16	19
9	1 (0.1%)	24	15	9	24
Total	938 (100%)	12968	12383	585	

The monthly *Toxocara* incidence rate showed a clear seasonal pattern (Figure 2), peaking during the winter and decreasing during the summer. Figure 2 also shows a decreasing trend in the incidence over the years.

Survival analysis

1. Time to "first" infection

Survival analysis for the time to FPI was based on 836 dogs with observations not ending on entry or beginning on FPI. These dogs accounted for a total of 8,783 dog-months at risk under observation during which 259 FPI occurred, resulting in an incidence rate of 2.9 FPIs per 100 dog-months (95%CI 2.6-3.3). Median time to FPI was 5 months (IQR 2-10).

The final multivariable Cox proportional hazards model for *Toxocara* FPI (Table 2) showed that the risk of observing a FPI was higher for dogs displaying coprophagic behavior or eating sand/soil, dogs ranging off-leash >80% of their walking time as compared to dogs ranging freely ≤20% of their walking time, dogs whose owners had noticed worms in their dogs' faeces, dogs fed with a commercial diet, and dogs with urologic or respiratory conditions. The risk of having a FPI was also significantly higher in winter and autumn as compared to summer, and it increased with increasing time since last deworming. Conversely, older age groups, having neurologic conditions, and being fed with a diet containing frozen raw meat had a lower risk.

Table 2. Results of the final multivariable Cox proportional hazards regression model for "first" T. canis *infection.*

	N dogs	N dog-months at risk	N observed FPIs	HR	95% CI		P-value
Age group							
0-12 months	202	636	42	Ref.			
1-7 years	575	5678	157	0.47	0.32	0.67	<0.0001
>7 years	250	2469	60	0.40	0.26	0.61	<0.0001
Sex							
Male	362	3702	113	Ref.			
Female	481	5081	146	0.93	0.72	1.20	0.580
Coprophagy							
No	475	4877	115	Ref.			
Yes	400	3906	144	1.36	1.05	1.77	0.021
Sampling season							
Summer	655	2332	38	Ref.			
Winter	741	2434	89	1.73	1.14	2.61	0.009
Autumn	564	1978	76	1.62	1.05	2.50	0.030
Spring	603	2039	56	1.28	0.81	2.01	0.287
Eating soil/sand							
No	740	7818	213	Ref.			
Yes	106	965	46	1.62	1.12	2.35	0.011
Following a commercial diet							
No	320	3081	68	Ref.			
Yes	567	5702	191	1.47	1.08	2.00	0.014
Following a diet containing frozen raw meat							
No	381	3377	131	Ref.			
Yes	494	5406	128	0.68	0.52	0.89	0.005
Having respiratory conditions							
No	822	8456	244	Ref.			
Yes	46	327	15	1.84	1.08	3.13	0.026
Having neurologic conditions							
No	819	8413	257	Ref.			

	N dogs	N dog-months at risk	N observed FPIs	HR	95% CI		P-value
Yes	40	370	2	0.21	0.06	0.82	0.024
Having urologic conditions							
No	816	8467	245	Ref.			
Yes	41	316	14	1.79	1.12	2.86	0.015
Excreting worms in faeces							
No	828	8688	252	Ref.			
Yes	10	95	6	2.26	1.16	4.42	0.017
Off-leash walking time (%)							
≤20	131	1325	29	Ref.			
20-50	260	2461	54	1.06	0.64	1.75	0.829
50-80	141	1337	40	1.30	0.76	2.23	0.341
>80	375	3660	136	1.79	1.13	2.83	0.013
Time since last de-worming (months)*	-	-	-	1.002	1.000	1.003	0.024

HR = hazard ratio, 95%CI = 95% confidence interval, FPI = "first" patent infection.
*Continuously time-varying variable let interact with the underlying time variable.

Table 3. Results of the final multivariable Cox proportional hazards regression model for T. canis *reinfection.*

	N dogs	N dog-months at risk	N observed RPIs	HR	95% CI		P-value
Age group							
6-12 months	49	110	30	Ref.			
1-7 years	202	2211	170	0.85	0.49	1.46	0.552
>7 years	96	926	84	0.87	0.47	1.62	0.663
Sex							
Male	121	1400	125	Ref.			
Female	162	1847	159	1.27	0.91	1.77	0.160
Coprophagy							
No	118	1234	84	Ref.			
Yes	170	2013	200	1.09	0.74	1.59	0.674
Sampling season							
Summer	237	777	57	Ref.			
Winter	220	612	95	1.68	1.15	2.46	0.008
Autumn	239	857	50	1.30	0.82	2.08	0.266
Spring	244	1001	82	1.43	0.96	2.14	0.079
Taking corticosteroids							
No	265	3036	263	Ref.			
Yes	25	211	21	2.38	1.09	5.19	0.029
Frequency of dog's faeces removal/disposal							
Never	32	351	48	Ref.			
Sometimes	160	1903	164	0.54	0.33	0.86	0.01
Always	89	993	72	0.54	0.32	0.91	0.02
Change in dog's main purpose/use							
No	280	3238	281	Ref.			
Yes	8	9	3	10.84	1.14	103.21	0.038
Having neurologic conditions							
No	279	3188	283	Ref.			
Yes	9	59	1	0.11	0.02	0.74	0.023
Having orthopaedic conditions							

	N dogs	N dog-months at risk	N observed RPIs	HR	95% CI		P-value
No	262	2891	260	Ref.			
Yes	45	356	24	0.55	0.28	1.06	0.074
Owner usually buys anthelmintic drugs at veterinary clinics							
No	134	1464	107	Ref.			
Yes	147	1783	177	1.52	1.10	2.11	0.011
Time since last deworming (months)*	-	-	-	1.003	1.001	1.005	0.000

HR = hazard ratio, 95%CI = 95% confidence interval, RPI = recurrent patent infection.
**Continuously time-varying variable let interact with the underlying time variable.*

Discussion

Longitudinal studies are better suited than cross-sectional studies to investigate

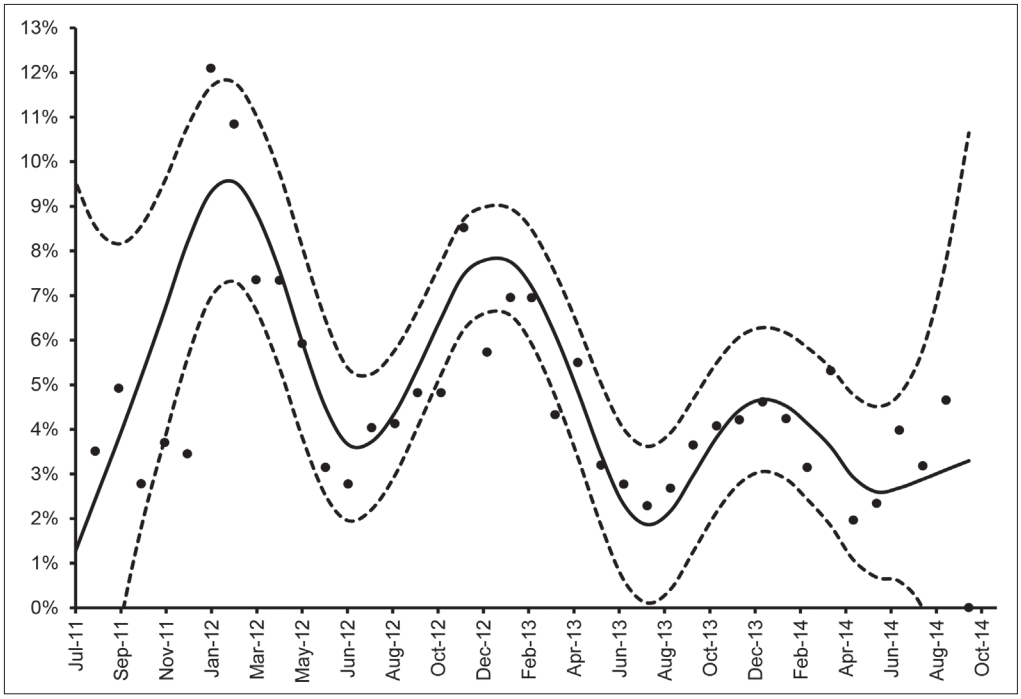

Fig. 2. Monthly T. canis *incidence (dots) over the study period (from July 2011 to October 2014). An optimized cubic smoothing P-spline function (solid line) and corresponding 95 % confidence interval (dotted lines) is fitted to the observed data.*

2. Time to reinfection
Time to RPI analysis was based on 281 dogs in which a FPI was diagnosed and from which subsequent samples were submitted. The corresponding incidence rate of 284 reinfections over 3,247 dog-months at risk under observation was 8.7 RPIs per 100 dog-months (95%CI 7.7-9.7). Median time to RPI was 9 months (IQR 3-16).

The multivariable Cox proportional hazards regression model for *Toxocara* reinfection (Table 3) showed that the risk of reinfection was significantly higher for dogs receiving corticosteroid treatment, for dogs whose main purpose/use was changed, and for dogs whose owners reported that they would usually buy anthelmintic drugs at veterinary clinics. The risk of reinfection was also significantly higher in winter as compared to the summer, and it increased with increasing time since last deworming. Conversely, the risk of RPI was significantly lower for dogs whose owners reported to sometimes or always collect and dispose of their dogs' faeces as compared to those who reported to never do that, as well as for dogs with neurologic conditions, and was borderline significant for dogs with orthopedic conditions.

events that can recur throughout an individual's life. Taking the limitations of coproscopical examination to diagnose patent *Toxocara* infections into account [30], our study reports an estimated prevalence of 4.5% dogs that shed *Toxocara* eggs, which is comparable with reported, mostly cross sectional, prevalences from current literature [14]-[16],[22],[23]. Monthly incidence ranged from 2% to 12%, peaking consistently during wintertime in all three years of follow-up. This finding was unexpected, as one would hypothesize that in a country like the Netherlands where seasons are well defined, dogs are walked outdoor for longer periods (and perhaps more often unleashed) during the summer as compared to winter because of the generally more favorable/pleasant weather conditions, and this would impose a higher risk for infection. Yet, similar seasonal patterns were noted by others [15],[31],[32]. Although no exhaustive explanation can be provided, it is evident that the winter peaks were consistently present in all three years of follow-up. The possibility that the observed seasonal pattern reflects more frequent deworming in summertime could be ruled out because the surveyed dog population was not routinely dewormed, as this was a condition for participation in the study. Wolves (*Canis lupus*), the far ancestors of dogs, are mono-estrus species that breed in mid to late winter, and the associated endocrinological changes might reactivate dormant *T. canis* larvae during that period as is known in dogs. It is likely that the change in day length is the stimulus of this breeding cycle. We speculate that in the co-evolution of the parasite and its definitive host, this phenomenon might have persisted even though household dogs do not necessarily show a well-defined seasonal breeding pattern any longer [33]. However, kenneled cyclic beagles were not at higher risk for developing a patent *T. ca-*

nis infection, which makes this hypothesis less likely [34]. An additional explanation might be that shorter walks and longer staying at home during wintertime may act as stressor, contributing to reactivation of dormant larvae. Reactivation of dormant somatic larvae is likely to be responsible instead of an increased risk of being re-infected by ingestion of infective eggs. A possible other stressor could be related to the intensive use of fireworks during the festive period in the Netherlands in the last months of the year. Accordingly, a recent study reported increased cortisol levels, a common indicator of stress, during winter in dogs [35]. Whatever the reasons might be, it is apparent that seasonality in *T. canis* egg shedding exists and needs to be considered in future studies, especially when these are performed cross-sectionally at one moment in time. Moreover, understanding the origin of this seasonal pattern is relevant for control, as any (blind) deworming would be more likely to be necessary during the coldest rather than the warmest months. Besides seasonality, monthly *Toxocara* incidence also tended to decline over time even though no blind deworming was applied. This may be explained by the aging of the cohort and by the loss of follow-up of some frequent shedders.

Most (67.9%) participating dogs were never diagnosed with a patent *T. canis* infection during the follow-up period, 17.5% dogs experienced only one infection, and 14.6% dogs experienced two or more infections, with a maximum of nine patent infections diagnosed in the same dog during a follow-up period of 24 months. Based on the observed frequency of infection, the average annual incidence rate was estimated at 0.54 patent infections per dog/year, which can be translated into one infection occurring approximately every two years among household dogs that are not (blindly) treated on a regular basis. Consequently, it could be said that the currently propagated 4-times-a-year anthelmintic treatment advice lacks evidence for dogs older than six months. Our data suggest that targeted treatments may be preferable over blind treatments. A two-step approach was applied in the longitudinal analysis. First, a survival analysis for identifying factors influencing the time to FPI was performed. Second, survival analysis was performed for identifying factors influencing the time to RPI. The time to FPI was measured from the moment of enrollment till the first diagnosed patent infection, without knowing when these dogs had actually experienced the previous patent infection before participating in the study. In general, similar risk factors for FPI were found in the present study compared to previous (cross-sectional) studies [13],[19]-[21], as well as to a previous cross-sectional study based on the same dog population, where only the first submitted sample of a dog was included, as in the present study [16]. These were young age, coprophagy, and proportion of walking time walking off-leash. An unexpected risk factor was the feeding of a commercial diet, while feeding frozen raw meat appeared to be protective. Though most of the *T. canis* larvae present in the meat will be killed by freezing it,

this is not always the case [36]-[38]. The assumption that raw meat may be a risk factor for *T. canis* infection is only valid if the meat in question contains dormant larvae. The origin of the consumed meat is therefore important to take into account, as meat from farms with a high level of biosecurity is highly unlikely to contain dormant larvae. Yet, previous research indicated that owners feeding raw meat to their dogs do not often know the origin of that meat (unpublished data). Explaining that commercial (bagged/canned) diets are a risk factor for patent *T. canis* infections is difficult. It is important to realize that this association might just be a spurious one due to a hitherto unknown confounder that was not accounted for in the analysis. We speculate that dogs receiving a commercial diet, which is usually easier for dogs to eat, might be more prone to the need of chewing/gnawing items, perhaps from the ground outside increasing the risk of infection. However, estimates were adjusted for "eating soil/sand" (included as covariate in the model) and were not influenced by the factor "eating items from the ground" (not significant). Interestingly, in the analysis of recurrent infections, factors related to diet were not associated with testing positive on *Toxocara* eggs. Eating soil/sand turned out to be a risk factor for FPI. This suggests that infective eggs are ingested, as coprophagy was controlled for in the analysis, or that eggs passively pass the gastro-intestinal tract after eating soil/sand. Normally, this would not lead to patent infection in adult dogs. However, it has been reported that infection with low numbers of eggs may sometimes lead to patent infection, as low numbers of larvae may pass undetected by the host's immune system during their hepatic-tracheal migration [12]. The observed effects of some health conditions on the risk of *Toxocara* egg shedding may be a reflection of the stress induced by the conditions themselves and/or by the decreased immune-competence that these conditions may entail. In contrast, having neurologic or orthopedic conditions stood out as a protective factor. Dogs with these conditions tend to be less active outside, thereby reducing the risk of acquiring a *T. canis* infection from the environment, which may oppose an effect of stress induced by these conditions.

The analysis of RPIs showed an incidence of 8.7 reinfections per 100 dog-months, more than 3 times the one for FPIs. This suggests that recurrent shedding of *Toxocara* eggs occurs more often in some dogs that for some reason are particularly prone to experience multiple patent *T. canis* infections. Such dogs may be called "wormy" dogs and, hence, should be a specific target for treatments. This group of "wormy" dogs is responsible for the majority (421 of 585 or 72%) of positive faeces samples (see Table 1).

Determining factors associated with (recurrent) infections in these dogs would therefore provide useful targets for control. The factors associated with RPIs found here showed some overlap with those for the FPIs. However, there were also some

interesting differences, which mainly concerned factors mirroring the immunological status of the dog. For instance, the administration of corticosteroids, known for their immunosuppressive action, resulted in a HR of 2.38 for experiencing a RPI. Sudden changes in the routine of the dog (i.e. main purpose or use of the dog), which may well lead to a temporarily suboptimal immune status due to stress, resulted in a hazard ratio of 10.84. The latter becomes even more plausible when having a closer look at the data (results not shown), as the owners whose dogs had their purpose changed mostly reported that their dogs had become hunting dogs. It is known that hunting activities can be quite stressful for dogs [39]. Previously identified risk factors for patent *Toxocara* infection may also be explained, to some extent, by (temporary) perturbations of the immune status, such as being kenneled [16]. This implies that dogs under periods of stress are at risk of becoming shedders of *Toxocara* eggs and should be targeted by anthelmintic treatment.

Cleaning up dogs' faeces by owners appeared to be protective for RPIs. Although such behavior in a dog owner is unlikely to be directly related to the risk of infection in the respective dog, it may mirror a general habit of disposing of dogs' faeces in the area where the owner lives, and therefore to a societal pressure to clean up dogs' faeces, possibly resulting in a generally less contaminated environment with *T. canis* eggs shed by dogs. Coprophagy was identified as a risk factor for FPI, as well as in a previous cross-sectional study [16], but it was no longer significant for RPIs. This suggests that *T. canis* eggs in the faeces of dogs showing recurrent infections are more likely to be eggs from an actual infection rather than eggs simply passing the gastrointestinal tract after ingestion of unembryonated eggs with the faeces of real *T. canis* shedders. In contrast, dogs incidentally shedding *Toxocara* eggs may often do so because of coprophagy. Coprophagy is a possible factor that can influence the outcome of coproscopical examinations and when performing such methods it should be considered when an animal tests positive [24],[25]. Finally, buying anthelmintics at the veterinary clinic was a risk factor for RPIs. This is hard to explain by simply looking at the biology of the parasite or the host. However, because anthelmintics in the Netherlands can also be purchased (sometimes for cheaper prices) at pet stores, internet, supermarkets, and department stores, owners buying anthelmintics at veterinary clinics do so probably because they happen to frequently visit the clinic for the health problems of their dogs, so this factor may simply mirror frequent veterinary care-seeking behaviors because of impaired health in the dogs.

Conclusions

Following a large cohort of dogs, all older than six months, up to 3 years without performing routine deworming in absence of a confirmed diagnosis revealed that approximately 68% of dogs never tested positive for *Toxocara* eggs. The overall incidence rate was 0.54 patent *Toxocara* infections per dog/year, meaning that a non-routinely treated dog is likely to shed *Toxocara* eggs once every two years, on average. However, the incidence rate of RPIs was much higher than that of FPIs, suggesting that there is a group of dogs particularly prone to recurrence of patent *Toxocara* infections. Dogs with RPI were responsible for the majority of positive faeces samples.
The identified risk factors for FPIs and RPIs indicate that there are two important aspects to consider when assessing the risk for a dog to acquire a *Toxocara* infection, the exposure to sources of infection and the failure of immunity. Indeed, both the likelihood of ingesting infective eggs/larvae and the possible evasion of immunity, perhaps by already present somatic larvae, should be taken into account when controlling *T. canis* infections in household dogs. Based on our study, this can be indicated by factors related to immune suppression, e.g. administration of immunosuppressive drugs or stress caused by underlying diseases or changes in routine, as well as factors related to higher chances of ingesting *T. canis* eggs from the environment, e.g. eating soil/sand or enjoying a high amount of off-leash walking time. Future modelling papers may benefit from studies that report on risk factors, especially when studied in a longitudinal set-up, so different scenarios can be tested by varying the exposure to different factors over time. Together with the observed peaks of *Toxocara* incidence during the winter months, our results suggest that blind deworming may be refined to become a more targeted deworming strategy based on the identified risk factors.

Consent to publish
All dog owners voluntarily participated in this study and agreed on publication of the anonymized data.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
RN and LM were involved in designing the study, analyzing the results, interpretation of the outcomes, and writing and revising the manuscript. RN was involved in coordinating the study and generating data concerning dogs and the questionnaires. LM was involved in constructing the survival analysis. LM and RN contributed equally

in this paper. JW and HP were involved in designing the study, critically revising the manuscript and in the interpretation of results.
All authors read and approved the final manuscript.

Acknowledgements
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, Charlotte de Wolf and Erwin Virginia for the administrative support. The owners participating in this study are acknowledged for supplying faeces and answering the questionnaires.

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Chapter 8
General discussion

Illustratie: Wim Hendrikx
Toxocara VIII
lijnets/aquatint 2016

Dogs, like other canids, are the definitive host of the roundworm *Toxocara canis.* In puppies, this parasitic worm can cause disease, but infection in adult dogs is usually asymptomatic. Nonetheless, during such an asymptomatic infection, some adult dogs can shed large numbers of eggs, and consequently contribute significantly to the environmental contamination with *Toxocara* eggs (Chapter 2, 6 and 7) (Claerebout et al. 2009; Overgaauw et al. 2009; Barutzki and Schaper 2011; Morgan et al. 2013). Humans can become infected with *Toxocara* by ingesting infective eggs from the environment and this can occasionally lead to disease due to larvae migrating through and settling in somatic tissues. Because of this, *Toxocara* infections are considered a world-wide public health issue (Woodruff 1970; Traversa 2012; Macpherson 2013; Overgaauw and Van Knapen 2013), necessitating control and preventative measures to lower the risk that environmental contamination with *Toxocara* eggs poses to humans. A major component of current *Toxocara* control is blind deworming of household dogs at varying degrees of treatment intensity, i.e. from treating several times a year to a "zero tolerance" policy. In an attempt to combine feasibility and effectiveness, this control policy has shaped the general advice to deworm household dogs at least four times a year, and dog owners are also urged to clean up their dog's faeces and to properly dispose of it (ESCCAP September 2010). Yet, the recommendations given by the European Scientific Counsel Companion Animal Parasites, ESCCAP, are not regulated by law, leaving it up to individual dog owners to comply or not. Moreover, although ESCCAP is an Europe-wide group of experts with sister councils in other continents propagating similar advices, there still remain many questions regarding their advice to control *Toxocara* spp. infections with respect to effectiveness and to what extent it is supported by scientific evidence. These issues are addressed in this thesis to contribute to a more evidence-based *T. canis* control policy, including a critical reflection on the attitudes of pet owners towards current control practices.

Blind deworming and prevalence of patent *Toxocara* infections

The currently propagated blind deworming advice for dogs is difficult to explain in view of the biology of *T. canis*. There certainly is something to say in favour of the advised blind deworming of all dogs, as every dog in which infection is curtailed before a patent infection has developed, means that there is less contamination of the environment with *T. canis* eggs. This can be an incentive to deworm as many dogs as possible at a predefined frequency. Indeed, it is assumed that nearly every dog carries a *Toxocara* infection, i.e. carries resting larval stages somewhere in their somatic tissues that can potentially be reactivated. Therefore, one may consider every dog to be at risk of shedding *Toxocara* eggs at some point in time. However, most cross-sectional studies report a prevalence of diagnosed patent infections of around 5% in household dog populations (Overgaauw 1997, Sager et al. 2006, Overgaauw et al. 2009, Claerebout et al. 2009, Barutzki 2011, Joffe et al. 2011, Paoletti et al. 2015), and this is in accordance with the results reported here (Chapter 2). Of note is that cross-sectional surveys are generally carried out in household dog populations for which blind treatments (at a frequency of four times a year) are propagated. However, even when dogs are monitored from several months to several years without being blindly dewormed (Chapter 7), the overall prevalence is still around 5%, varying from 2% to 12% and peaking during wintertime. Therefore, if blind deworming of all household dogs older than 6 months is propagated, circa 88-98% of these dogs will not have a patent infection at the moment of treatment, nor will an advice to do so four times a year on a voluntary basis have any apparent effect on the prevalence of patent *Toxocara* infections. Hence, it can be questioned whether blind deworming of all dogs is the most efficient and acceptable approach. Anthelmintic drugs are, in their registered dose, only effective against adult intestinal worms and actively migrating larvae, but they are not effective against the resting stages within somatic tissues. Additionally, we showed that a substantial proportion of identified cases of *Toxocara* egg shedders can be explained by passive passage of *Toxocara* eggs due to coprophagy, meaning that they do not arise from a true patent infection (Chapter 5). Consequently, the true prevalence of patent *Toxocara* infections in adult dogs might be much lower than the one derived from a mere look at the presence of *Toxocara* eggs in single faeces samples.

The above implies that by far most anthelmintic drugs given to non-juvenile household dogs are given to animals in which these drugs are unlikely to have an effect, simply because there are no worms in these dogs' intestines. According to the rules of conduct of Good Veterinary Practice, which are defined by the Federation of Veterinarians of Europe (Federation of Veterinarians of Europe 2002), treating an animal requires a proper diagnosis before starting the treatment, followed by an evaluation of the ef-

fect of a treatment after or during the period of treatment. One of the set exceptions to this rule concerns "*the routine preventative anti-parasite treatments in companion animal practice*". However, when *T. canis* is concerned, is it really "preventative" to deworm a dog blindly four times a year if in the majority of dogs, at any given point of time, there are no worms to treat? Clearly, in most cases this exception to the rules of conduct allows unnecessary treatments without proper diagnosis and evaluation of efficacy, which is in stark contrast with the general idea of Good Veterinary Practice.

Duration of drug activity and frequency of deworming

Ideally, anthelminthic treatment aims at controlling, curing or preventing parasitic infection in an animal, thereby preventing or reducing environmental infection pressure and/or curing or preventing that an infection will lead to clinical disease. As, for *Toxocara* infections in non-juvenile dogs, this last point is usually not the case, this will be hard to ascribe to treatment. Considering the prevention of patent infections, anthelmintics registered for dogs in the Netherlands have a duration of activity varying from several days to one month. According to the answers on the questionnaire, as described in chapters 2 and 7, the anthelmintic drugs that were used most frequently by the participating dog owners before they enlisted in the project, were short-acting ones (unpublished data), with a duration of effect of a few days. If this reflects the situation of all dog owners in the Netherlands, then, at least theoretically, a window of eight months a year is left open during which a dog may develop a patent *Toxocara* infection if the deworming advice of four times a year is followed. This is based on a generally assumed prepatent period of between four to six weeks (Parsons 1987, Fahrion et al. 2008) following either ingestion of infective eggs, or of larvae from devoured paratenic hosts. With the use of anthelmintics that are active for a month, this window will be reduced to four months, but still leaves a period in which patent infections may develop. Therefore, treatment frequency is recommended to be intensified, up to on a monthly basis, in so-called risk situations like "a pet living in a family with small children and common use of a garden (or similar situations)" (ESCCAP September 2010). This example shows the possibility of deploying a more tailor-made deworming advice and underlines a need for identifying and mapping risk factors for patent *Toxocara* infections in final hosts, as we did in chapters 2 and 7 for dogs and chapter 4 for cats. Having said the above, there remain situations in which the duration of the prepatent period is still unclear.

Variable prepatent period

In general, the prepatent period is considered to be the period from ingesting an infective stage until the host starts shedding eggs/larvae/(oo)cysts into the environ-

ment. However, it is widely accepted that the majority of, if not all, adult dogs already carry resting larval stages. These larvae can reactivate under the influence of immunosuppressive events, such as pregnancy, disease, medication, or stress, leading to a patent infection (Chapter 7). How reactivated somatic larvae that complete the hepato-tracheal migration fit within the above described treatment recommendations is, except for the period following pregnancy, unclear. For example, reactivated larvae in a dog may develop into adult worms seemingly within a shortened prepatent period following anthelmintic treatment. However, the prepatent period will actually have been longer than four to six weeks as the larvae got encapsulated some time before, "unreachable" for the anthelmintic, and were somehow reactivated after treatment to finish their migration route. If the reactivation is due to impaired immunity following e.g. a diagnosed illness or use of immune-suppressive medication (Lloyd et al. 1981), a custom-made *Toxocara* control advice may be communicated with the owner as suggested in chapter 7. However, recognizing impaired immunity is not always easy if the dog does not show overt signs of disease. Identification of dogs without a disease history, but with a less functional immunity against patent *Toxocara* infections is possible through coproscopical examination. By performing this diagnostic aid, frequent shedders can be identified and subjected to a stricter deworming schedule. A major obstacle, however, is the fact that the costs of coproscopical examination cannot compete with those of anthelmintic treatment. Moreover, after a positive diagnosis, an owner has to pay for both anyway. Introducing a "preventative care-package" in veterinary clinics could help to facilitate the implementation of routine coproscopical examination to decide on the necessity of anthelmintic treatment. Such a package could combine routine physical examinations, dental care, nutritional advice, vaccinations and parasite control. Performing regular coproscopical examination will gain valuable information about the susceptibility to patent worm infections of individual dogs. On the other hand, implementing a sound faecal examination schedule is probably just as difficult as implementing a sound blind treatment regimen. Examining faeces for *Toxocara* eggs four times a year will also result in large windows in the control of *Toxocara*, as a negative result cannot be interpreted as absence of actively migrating somatic larvae or pre-adults (not yet reproductive stages) in the intestines. Thus, although periodical routine faecal examination would lead to useful epidemiological information and identification of frequent shedders, it does not fully meet the goal of preventing *Toxocara* egg shedding as might/will be required for public health reasons.

Compliance to recommendations

As mentioned above, from a public health perspective, the most logical blind deworming frequency might be the one based on the regular prepatent period of *T.*

canis (Parsons 1987, Fahrion et al. 2008). This would mean deworming all dogs every four to six weeks, meaning 9-13 times a year, which conforms to an absolute zero-tolerance policy, even though this policy would still not cover immuno-compromised dogs that may shed eggs in unexpected shorter intervals. The question is whether dog owners would accept such a zero-tolerance policy and to what extent this would result in a reduction of the public health hazard of environmental contamination with *Toxocara* eggs.

As reported in chapter 2, compliance to the current recommendation of treating four times a year is a long way from 100%. About 11% of the dogs did not receive any anthelmintic treatment by the owners and the largest proportion of dogs (41%) was dewormed once to twice a year. Only about 16% of the dogs were dewormed at least four times a year. By deworming four times a year at a compliance of 90%, according to our model the contribution of household dogs to the environmental contamination would be reduced by about a third (Chapter 6), which implies that the contribution of household dogs to *Toxocara* eggs in the environment would still remain substantial. It is hard to predict whether these fewer *Toxocara* egg-shedding dogs, under such a scenario, would lead to a significant decline in human infections. Integrating our model with a recently published model that assessed the relation between environmental contamination and seroprevalence in humans (Kanobana et al. 2013) could be useful for assessing the effect of deworming dogs on the seroprevalence in humans.

The poor compliance to the general advice raises some questions about if and how these owners are informed about the issue of routine anthelmintic treatment of their dogs. This is even more reflected in the answers about the main reason for routinely deworming dogs, in which only 14% of the owners recognized public health as the most important reason and 7% mentioned a combination of dog's health and public health. The majority of the owners (68%) mentioned "the dog's health" as the most important reason for regular anthelmintic treatment. When "the dog's health" is the main incentive for owners to consider anthelmintic treatment, in combination with a roundworm that rarely causes disease in non-juvenile dogs, it is not surprising that the compliance is less than desired. In a previous publication it has been demonstrated that more than half of the dog owners that visited a small animal clinic in the Netherlands did not follow the recommended deworming advice (Overgaauw et al. 2009). Despite efforts being made, for example by ESCCAP, on informing owners and making them aware of the existence and public health risks of this parasite in dogs, owners are either insufficiently aware of, or not prepared to act on the recommended deworming advice. In both cases, changing the mindset of owners to comply better to recommendations will be difficult.

Still, dogs' contribution to the environmental contamination will likely be reduced with about 50% if only half of the dog owners would deworm 12 times a year. When blind deworming four times a year is the advice, it is not surprising that the expected reduction resulting from a better compliance remains far from this halving in contribution. Under current conditions, a 50% compliance to deworm a dog four times a year is not to be expected, while its effect may be considered marginal. This, therefore, may lead to the conclusion that intensifying the advised frequency of deworming might be more successful than just improving compliance. However, when the recommended frequency of deworming household dogs is intensified from 4 to 12 times a year, this probably will also affect the compliance of dog owners to such a deworming advice or deworming dogs in general, especially in view of the current lack of knowledge of dog owners about this matter. It is difficult to predict whether compliance will increase or decrease following an advice to treat on a monthly basis, and therefore it will be difficult to predict how this would affect the relative contribution to the environmental contamination (Chapter 6). Scientific evidence on changing human behaviour is lacking for comparable situations where treatment of a third party (in this case the dog) is advised, not for guarding the health of that third party or personal health of the acting party (the owner), but for public health in general. One recent publication on *Toxoplasma gondii* in household cats comes close to such a situation. This paper reported that 85% of the cat owners visiting a veterinary clinic in the Netherlands would be willing to pay some amount for vaccination of their cats against *Toxoplasma* (Opsteegh et al. 2012). Though only intentional, this suggests a much better compliance than our results show for anthelmintic treatment. This difference in compliance may be explained by several aspects. First, it is easier to express an intention than to actually follow-up on it. Yet, 85% does suggest that compliance would indeed be much higher. Second, vaccination is done by a professional, whereas anthelmintics are over-the-counter products to be administered by owners themselves, which involves an extra effort. Third, *Toxoplasma* is probably associated with a much better defined and known burden of illness in humans, related to pregnancy and congenital infections, than *Toxocara*.

An intensification of the recommended deworming frequency needs to be accompanied by compelling arguments from retail points and professionals. Unfortunately, so far, communication has not been sufficient to invoke an acceptable willingness among dog owners to comply with the advice. A reason for this could be the quality of the information provided at retail points for anthelmintics, including veterinary clinics. In an older study among Dutch veterinarians, it was reported that the knowledge of clinicians about the recommended treatment schedule to control *Toxocara* infections in pets was not sufficient (Overgaauw and Boersema 1996). Another study performed in Canada also noted a suboptimal level of knowledge for providing prop-

er deworming advices in veterinary clinics (Stull et al. 2007). Clearly, there is a need for improving the information provided by veterinary professionals at all levels, as well as by people working in pet shops, beyond a simple advice about how often one should treat blindly. This should be accompanied by ways to convey that information in an easy and comprehensible way for pet owners.

Other sources of *Toxocara* eggs in the environment

Apart from compliance of dog owners to the recommended treatment regimens, the effect of the current deworming advice on the environmental contamination with *Toxocara* eggs also depends on other factors. An important additional factor is the fact that non-juvenile household dogs are not the only source of *Toxocara* eggs in the environment (Chapters 3, 4, and 6). Another species of *Toxocara* that contributes to the zoonotic burden is *Toxocara cati* (Fisher 2003), of which the eggs are shed by cats. The number of household cats exceeds the number of household dogs (HAS den Bosch and Utrecht University 2015). Predation is probably a very common behaviour in cats and more so than in household dogs. Cats prefer burying their faeces, often in gardens and sandboxes (Uga et al. 1996). Moreover, there is a large population of stray cats living in the Netherlands (Neijenhuis and Van Niekerk 2015), while there are no stray dogs. All these factors strongly suggest that cats can be considered at least as important as dogs as a source for human toxocariasis (Fisher 2003, Morgan et al. 2013). The outcome of our model used in chapter 6 suggests that cats as a whole contribute more to the general environmental contamination with *Toxocara* eggs than dogs. Additionally, fouling of the environment that does not belong to the property of an owner of an animal is more or less accepted for cats, but not for dogs. Besides, most dog owners will be aware of some kind of social pressure and one generally considers cleaning up dog faeces as proper behaviour. However, it is very unlikely that cat owners, when their cats defaecate outside, are physically present at the location of defaecation, let alone that they feel any pressure to clean up afterwards.

Red foxes (*Vulpes vulpes*) are also members of the family of Canidae and they are also known to shed *T. canis* eggs in much higher prevalence than household dogs (Chapter 3) (Borgsteede 1984, Saeed et al. 2006). Foxes are endemic in the Netherlands with an estimated population density of 0.5 to 4.0 foxes per km^2 (Chapter 3). The living environment of foxes and humans is not as closely shared as that of humans, dogs and cats. However, some recreational parks or forests do belong to their territory and foxes are regularly seen in areas of high human population densities (Van Gucht et al. 2010, Muijen 2014).
Another canid species, which is also endemic in the Netherlands, yet probably still in small numbers, is the raccoon dog (*Nyctereutes procyonoides*). Little is known

about the numbers living in the Netherlands, the prevalence of patent *Toxocara* infections in this species, or about their distribution throughout the country. However, it is thought that this species is very adaptive, lacks predators preying on them, and reproduction in the Netherlands has already been reported. It is known that this species is emerging from the North Eastern part of the Netherlands, though the spreading might be blurred because they are also kept as pets in unknown numbers (Mulder 2011). This makes this species a topic for future studies on *Toxocara* as the lack of information at this point makes it impossible to estimate their contribution to the environmental contamination, although it currently may be negligible.

So overall, even when all dog owners would comply to deworm their dogs in an intensified scheme of twelve times a year, it would result in a maximum reduction of about 40% in the total environmental contamination with *Toxocara* eggs. In urbanized areas, where the vast majority of the Dutch population resides, the maximum reduction will probably be about 15%. Despite these relatively low levels of possible reduction in contamination, it is worthwhile to pursue a situation where household dogs are not contributing at all. The best efficacy of advised interventions is to be expected in the group of household dogs, compared to cats, let alone foxes, because dog owners have probably more control over their animal's defaecation behaviour than owners of a free roaming household cat. For instance, it is easier to prohibit household dogs to enter into public parks. However, it should be kept in mind that if these areas are accessible for cats and foxes, the effect on contamination of the park with *Toxocara* eggs due to prohibiting access for dogs should be put into perspective of the contribution of the other host species.

Risks of full compliance to treatment recommendations

As mentioned above, deworming dogs will lead to a reduction of environmental contamination with *Toxocara* eggs. And, for public health reasons, it might make sense to advocate a "zero tolerance policy" involving anthelmintic treatment of as many dogs as possible 10-12 times a year.

However, very intensive treatment strategies have led to widespread anthelmintic resistance in horses and small ruminants (Kaplan and Vidyashankar 2012). The speed of the development of anthelmintic resistance of nematodes is partly dependent on the size of the worm population that will not come in contact with an anthelmintic substance, the so called "refugia". Because of the relatively low compliance of dog owners to the current deworming advice, combined with the presence of a sylvatic population of foxes, the travel movements of dog owners with their dogs, and the number of different anthelmintic substances that is available, the refugia is expected

to be large. Therefore, resistance of roundworms in household dogs, if at all present, is not expected to spread fast. However, there is evidence of resistance in other nematode species of companion animals. A high level of resistance against pyrantel was reported for the hookworm *Ancylostoma caninum* in Australia (Kopp et al. 2007). Recently, reduced sensitivity was reported for *Dirofilaria immitis* in the United States (Bourguinat et al. 2015). For *D. immitis* the preventative treatment advice is on a monthly basis. Resistance to one product generally develops sooner if that product is used predominantly. As mentioned above, from our survey reported in chapter 2 it appears that one type of short-acting anthelmintic drug (containing milbemycin) is used most frequently among dog owners (unpublished data). Moreover, efficacy of blind anthelmintic treatment in companion animals is not routinely checked in the Netherlands. And finally, from horse and ruminant practice it is known that if resistance emerges, it usually becomes noticed for the first time when already showing overt inefficacy. This pleads for the use of diagnostics before, but also after, treatment. The alternative of only deworming those dogs that test positive on coproscopical examination, might lead to a less strict selection pressure, depending on when a patent infection is detected. A disadvantage of this strategy can be that if a patent infection is only detected days or weeks after patency started, the dog may still have shed numerous *Toxocara* eggs into the environment.

Environmental pollution with residues of anthelmintics can also raise questions about the advice to deworm twelve times a year, because of potential threats to ecosystems, and maybe even an effect on the free-living stages of parasitic worms. To the knowledge of the author, this last topic has not been investigated. Studies have so far mainly focussed on anthelmintic substances that are commonly used in livestock. These studies do report on toxicity of residues of anthelmintics in faeces, having effects on arthropods and non-parasitic nematodes (McKellar 1997, Kolar et al. 2008, Suarez et al. 2009, Beynon 2012, Horvat et al. 2012). These residues can have a long half-life time in the environment, e.g. between 93 and 240 days for ivermectin (Halley et al. 1989), which is not used as anthelmintic for dogs in the Netherlands, but is used frequently in areas in which heartworm is endemic. This environmental pollution will be less if targeted treatment would be introduced. Therefore, this is a topic that needs attention, especially when a monthly deworming advice is considered for dogs.

Another question, already briefly addressed, concerns the effect on the compliance of dog owners to a change in the propagated deworming advice involving tripling the deworming frequency. There is a possible risk that this will have an adverse effect on the compliance. From research on compliance to self-medication it is known that eliminating unnecessary medications, and reducing the frequency of treatment increases the compliance (Rudd 1995, Kendler et al. 2004, Cramer et al. 2006).

Though this is not really comparable to treating one's dog in intensified intervals, it at least suggests that increasing the advised frequency of deworming can be of influence on the compliance rate to the advice. Besides the input from epidemiologists, public health professionals, medical doctors, and veterinarians, this question probably needs the input of specialists in modification of human behaviour to guide and optimize the desired change in behaviour of dog owners. Collaboration of these different disciplines in a "One Health *sensu lato*" effort should lead to increased social/peer pressure emphasizing and enhancing the responsibility of owning animals with respect to the environment and public health.

Pointers for improvement

It is clear that there are legitimate concerns about anthelmintic treatment being the main solution for controlling patent *Toxocara* infections in dogs to reduce the incidence of human toxocariasis. Therefore, alternative or additional measures should be seriously contemplated. One of the most obvious alternative or conjunctive measures to anthelmintic treatment is removing dog faeces and disposing of it properly, which should be relatively easy to stimulate and enforce if well facilitated and promoted by veterinarians, pet shops, dog training centres, dog groomers, breed clubs, and national and local government.

Possible exposure of humans to infective *Toxocara* eggs may depend on the defaecation locations of dogs and on the intended use of these locations. The direct effect of reducing the number of *Toxocara* eggs ending up in the environment on the number of infected humans will probably be lower in an area not used for recreational purposes or where dog faeces is cleaned up timely and on a regular basis, than in, for example, a picnic area with less hygienic maintenance. Preventing dog faeces from contaminating the immediate environment of humans is a feasible alternative for deworming and can be executed by, for example, not allowing dogs in recreational areas and/or facilitating cleaning up of dog faeces in recreational areas where dogs are allowed. The current compliance level to cleaning up dog faeces is reported in chapter 6, which showed that there is definitely room for improvement. Of all dog owners, 42% to 74%, depending on the level of urbanization, never or rarely cleans up their dogs' faeces. In a study from Northern Ireland, factors influencing owners' attitude towards cleaning up dogs' faeces were assessed (Wells 2006). These included social economic status, gender, and leash use. A study from Portugal suggests that a civic component, linked to social pressure, is involved (Matos et al. 2015). Improving compliance to clean up faeces, can be supported by the fact that dog faeces on sidewalks, in parks, and in playgrounds is considered one of the most irritating aspects in daily life (Atenstaedt and Jones 2011, Derges et al. 2012). Only a few studies

reported on this topic and provided suggestions to improve dog owners' compliance to cleaning up their dogs' faeces. Key factors in parks, associated with the presence of dog faeces, were availability of bins to get rid of the collected faeces, path morphology, being visible to others and location (Lowe et al. 2014).

The effects of improving the knowledge of dog owners, but also of workers at retail points selling anthelmintics, are expected to be beneficial. As pointed out earlier, a Dutch study reported on the insufficient knowledge of veterinarians working in veterinary clinics (Overgaauw and Boersema 1996). Information packages about *Toxocara* infections in pets were sent by mail to inform veterinarians. Infomercials on radio, articles in pet related magazines, and television time in kid shows were used to inform the general public. The campaign did result in an improvement of the knowledge of veterinarians at certain points concerning *Toxocara* and deworming strategies. Nonetheless, the conclusion was that, in general, the knowledge of veterinarians about *Toxocara* and how to act on this in practice, was still insufficient. In a study from Belgium, where owners were stimulated to visit a veterinary clinic for a free check-up of their animal, a very low level of systematic preventative care against worms was reported (Diez et al. 2015). Health screening was mentioned as an opportunity to improve this. For the current situation in the Netherlands it is not expected that the situation changed significantly since the campaign launched two decades ago, at least not in view of our results (Chapter 2). Clearly, continuous (re-)education of professionals and the general public should be an integral part in campaigns on *Toxocara* control. Stalsby Lundborg et al. (2014) used the "stages of change" theory to explain human behavioural changes with respect to the use of antibiotics. Along similar lines and based on questionnaire results (Chapter 2), attempts to change dog owners' behaviour appear to be stuck in "the pre-contemplation phase" (unaware of the problem, not thinking about change) and "the contemplation phase" (considering change in the future, but not ready for action). To expect real changes, progression towards "the preparation/decision stage" should be promoted. Up to now, campaigns on controlling *Toxocara* centered around deworming dogs without an immediate apparent incentive for dog owners and unidirectional dogmatic education. Compliance to recommendations is likely to benefit from active participation of dog owners to shape these recommendations (Abood 2007). This is already supported by using a simplified decision tree for owners to determine which risk group their dog fits into (ESCCAP November 2014). In short, 'ownership of the problem' should extend to within the responsibility of the dog owner and not be restricted to veterinary and medical professionals.

Another interesting result was the apparent seasonality of *Toxocara* egg shedding (Chapter 7). It shows that without changing or intensifying the number of advocated

anthelmintic treatments or investing in improving the compliance, efficacy can be improved by concentrating treatments in the season where most egg shedding occurs. Seasonal patterns are mentioned in other studies too (Kirkpatrick 1988, Nolan and Smith 1995, Sowemimo 2009, Barutzki and Schaper 2011), but this has not resulted in an adapted advice for blind deworming. If the advice states four times a year and the variable compliance is generally accepted, it can be emphasized that the period from October until April is most important to comply.

A last major point of interest to improve *Toxocara* control, concerns the occurrence of recurrent infections (Chapter 7). Such infections appear to be mainly present in the so called "wormy animals", and these animals contribute by far the most to the environmental contamination with *Toxocara* eggs. Identifying these animals by investing in coproscopical diagnosis, and by using a decision tree based on known risk factors is expected to improve efficacy without a need to improve compliance to deworm dogs in general. Owners of "wormy dogs", with frequently visible worms in the faeces, are likely more willing to apply regular anthelmintic treatments than owners whose dogs never show any signs of carrying a worm infection. The principle of targeted anthelmintic treatment based on diagnosis and risk assessment is increasingly used for both horses and small ruminants, and not without success. Several reports conclude that, following implementation of targeted treatment strategies, usage of anthelmintic drugs reduces substantially without increases in disease incidence or lowered productivity of animals (Kenyon and Jackson 2012, Menzel et al. 2012, O'Shaughnessy et al. 2015, McBean et al. 2016). Although incentives for treating companion animals differ from those in horse and ruminant practice, a more targeted treatment strategy probably will have the beneficial effect of getting the dog owner involved in the decision making process. A subsequent effect may be that where dog owners may judge (veterinary) advice strictly as a 'sales talk', now they may experience a more interactive decision-making process on whether or not to treat their dog as a real interest in their animals, which may help increase adherence to the resulting decision.

Human toxocariasis in relation to *Toxocara* control in dogs

So far, the discussion focused on control of *Toxocara* infection in dogs, under the premise that any chance of human toxocariasis should be eliminated. However, one may ask whether the burden of illness in humans is large enough to propagate a zero-tolerance control policy in dogs, which, strangely enough, is left to voluntary efforts of dog owners themselves, stimulated by veterinary or other advice or not. As mentioned in the introduction of this thesis, diagnosis of toxocariasis in humans is challenging (Fillaux and Magnaval 2013). Studies are commonly restricted to individ-

ual case-reports and serological screening of suspected toxocariasis patients (Good et al. 2004, Goto et al. 2007, Rubinsky-Elefant et al. 2010, Pinelli et al. 2011, Ahn et al. 2014) or in the general population (Mughini-Gras et al. 2016). Although this gives an idea of human exposure to *Toxocara* and emphasizes that this is not without risk of an ensuing disease, it still does not provide a clear picture of the "burden of illness" due to human *Toxocara* infections (Smith et al. 2009). It is also not exactly clear how infection, with or without disease, relates to the environmental contamination with eggs of *Toxocara.* Of course, ingesting infective eggs of *Toxocara* by humans, is, in general, assumed to be the most important infection route. So, do we need to eliminate any environmental contamination, or is there some level which may be considered acceptable in terms of risk for human toxocariasis? In the Netherlands, seropositivity is common in the general population, but it has not been related to levels of environmental contamination as such, rather indirectly using risk factor analysis (Mughini-Gras et al. 2016). A study from Brazil reported that public squares, frequently visited by dogs, contributed positively to seropositivity in children visiting those squares. Seropositive children played at squares where the contamination level was higher than 1.1 eggs per gram of sand (Manini et al. 2012). A study from Poland related persistence of seropositivity in children after treatment to reinfection by assessing the environmental contamination with *Toxocara* eggs in their living environment (Zarnowska et al. 2008). Another Brazilian study reported no association between the level of environmental contamination and seropositivity in children (Mattia et al. 2012). At best, results are difficult to interpret and may even be conflicting. For pathogens like *Giardia duodenalis* and *Cryptosporidium* acceptable levels of contamination of drinking water have been defined (Smeets et al. 2009). It might be worthwhile to investigate the same for *Toxocara* infections, which may assist in determining the level of effort required for *Toxocara* control in household dogs.

Concluding remarks

A few issues remain concerning both *Toxocara* control and implications resulting from the studies carried out within the scope of this thesis. First, the current deworming regimen for dogs is focused on *T. canis* infections and its associated public health risks. However, if other helminths are considered as well, the discussion on control policies has to consider control requirements for these other infections as well, changing and possibly complicating what should be the overall general advice. For example, emerging infections like *Echinococcus multilocularis* (Takumi et al. 2008), *Angiostrongylus vasorum* (Van Doorn et al. 2009), and perhaps in the near future also *Dirofilaria immitis* (Genchi et al. 2009), all pose either public health threats and/or may cause significant disease in the dog itself. Clearly, it may change and intensify advocated treatment frequencies to which owners probably are more

willing to comply to (e.g. because of the risk of heartworm disease in the dog). But, it also may have inadvertent medication consequences as anthelmintic drugs are more and more combination products. For example, to treat against the tapeworm *E. multilocularis* praziquantel is the drug of choice, which generally is not effective against nematodes. However, it is not available as a monovalent product in the Netherlands and is only available with another ingredient that is active against nematodes. Therefore, one also should reflect on the need for control of other parasitic infections, with their own dynamics, and how this might influence *Toxocara* control.

Second, all owners of dogs and cats participating in our studies, did so voluntarily. This may have created some selection bias as participants were asked to put in quite some effort into submitting faeces samples and entering a questionnaire on a monthly basis. However, in view of the results which generally conform to those found in many other studies, the wide distribution of participants over the entire country, the range of dog breeds involved, and the range of dog ages involved among other things, we feel confident that the results and conclusions are valid for household dog populations in general, both in the Netherlands as abroad.

Third, there still is a lot to elucidate about the course and intensity of patent infections in adult dogs. The widely accepted methodology to detect *Toxocara* eggs in the faeces has a detection limit, as all other coprological techniques, which may allow for a proportion of false negatives (Becker et al. 2016). It would be interesting to investigate the range of patent *Toxocara* infection intensities, including temporal fluctuations herein, occurring in the field with a technique that would not allow false negative results. It also would provide more quantitative data on the numbers of eggs that non-juvenile household dogs actually shed into the environment. Nonetheless, the technique used in this thesis is the same technique, give or take a slight modification, as has been used in the majority of studies world-wide. Therefore, results can be easily compared to those other studies in terms of *Toxocara* prevalence.

In conclusion, this thesis provides a critical reflection on some important topics related to patent *Toxocara* infections and the related accompanying current deworming advice propagated in the Netherlands and Europe as a whole. The major results are: i) *Toxocara* eggs are shed by approximately five percent of non-juvenile household dogs, which conforms to results obtained elsewhere. Prevalence of egg shedding appears to have not changed over the last decades; ii) moreover, overall prevalence was not higher in untreated dogs monitored from several months up to several years, suggesting the inefficacy of advocated blind treatments to lower *Toxocara* prevalence in household dogs; iii) “wormy” dogs, having recurrent patent infections, are responsible for the majority of positive faeces samples, supporting the use of diagnostics in

identifying animals that are at a high risk of shedding eggs; iv) the knowledge of and compliance to the advised deworming regimen and cleaning up dog faeces of dog owners leaves much room for improvement; v) a quantitative estimate was made on the contribution of dogs to the overall environmental contamination with *Toxocara* eggs relative to other final host species (cats and foxes), which amounts to approximately 40%.

Based on the results four major topics for improving *Toxocara* control are suggested, involving compliance to cleaning up dog faeces, continuous education of and involving dog owners in the decision to treat their dog, taking into account the seasonal effect in *Toxocara* egg shedding and focusing on "wormy dogs" as these are the major contributors to environmental contamination.

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Nederlandse samenvatting, English summary, Dankwoord en Curriculum vitae

Illustratie: Wim Hendrikx
Toxocara IX
lijnets/aquatint 2016

NEDERLANDSE SAMENVATTING

Achtergrond

Toxocara canis, de spoelworm van de hond, is de meest voorkomende parasitaire worm bij honden in Nederland. Bij volwassen honden veroorzaakt deze worm meestal geen tot weinig verschijnselen. Bij puppy's kan de worm echter tot ernstige ziekte leiden. Ook bij de mens zijn ziektebeelden bekend die toe te schrijven zijn aan rondtrekkende larven in het lichaam, het is dus een zoönose. Vanwege het vermogen om ziekte te veroorzaken in jonge dieren en met name ook vanwege het zoönotisch potentieel is *T. canis* bepalend voor het in het Nederland uitgedragen ontwormingsadvies. Binnen dit advies worden dieren tot de leeftijd van een half jaar in een hogere frequentie ontwormd dan oudere dieren. Over het ontwormen van pups bestaat weinig discussie. Dit heeft te maken met het feit dat pups al voor de geboorte in de baarmoeder geïnfecteerd kunnen worden en na de geboorte ook via de moedermelk. Wanneer de pups niet meer bij de teef drinken kunnen ze zich, net als honden die ouder zijn, alleen nog maar infecteren via opname van infectieve eieren vanuit de omgeving of via het eten van geïnfecteerde prooidieren. Al deze genoemde infectieroutes kunnen tot gevolg hebben dat er zich in een pup een zogenaamde patente infectie ontwikkelt. Dat betekent dat de pup zelf ook enorme aantallen eieren, geproduceerd door volwassen wormen in het darmkanaal, met de ontlasting uit gaat scheiden. In verse ontlasting zijn deze eieren nog niet direct infectief. Zij moeten eerst nog een aantal weken in de omgeving rijpen voordat zij infectief worden. Deze infectieve eieren kunnen weer tot nieuwe infecties bij (jonge) honden leiden. De larve die uit een eitje komt, moet wel eerst een hele trektocht door het lichaam van de jonge hond maken en komt dan in de longen terecht, wordt opgehoest en doorgeslikt om vervolgens in de darm volwassen te worden. Aangenomen wordt dat de meeste honden ergens gedurende de eerste zes levensmaanden een zogenaamde 'leeftijdsresistentie' zullen opbouwen. Wanneer een hond ouder dan zes maanden zich via de opname van infectieve eieren infecteert, dan leidt dit niet tot volwassen wormen in het maag-darmkanaal van de hond, maar loopt de infectie ergens in het lichaam vast. De meeste honden die ouder zijn dan zes maanden zullen dus geen patente infectie meer ontwikkelen. Vandaar dat er vragen bestaan bij eigenaren en dierenartsen of alle honden ouder dan zes maanden wel even vaak moeten worden ontwormd. Wanneer infectieve eieren echter worden opgenomen door andere diersoorten, inclusief de mens, dan leidt dit niet tot volwassen wormen in de darmen, maar loopt de larve die uit zo'n eitje komt ergens in het lichaam vast. Hier kan dit, afhankelijk van de plaats waar zo'n larve uiteindelijk terecht komt, tot verschijnselen leiden.

Omdat ook de mens geïnfecteerd kan worden, met in zeldzame gevallen complicaties tot gevolg, wordt geadviseerd om elke hond ouder dan zes maanden minstens vier keer per jaar ontwormen. Het is namelijk bekend dat sommige honden, ondanks die 'leeftijdsresistentie', op een gegeven moment toch een patente infectie kunnen ontwikkelen en dus eieren uit gaan scheiden. Een deel van deze gevallen is te verklaren. Bijvoorbeeld bij teven die een nestje hebben. Hier worden de in het lichaam vastgelopen larven geactiveerd en beginnen weer door het lichaam rond te trekken. Op deze manier kunnen zij de pups al in de baarmoeder infecteren en na de geboorte ook via de melkklieren van de teef. Wanneer de teef bij het verzorgen van de pups en het schoonmaken van het nest de ontlasting van de pups op eet, krijgt zij ook larven binnen die in de ontlasting van de pup kunnen zitten. Een infectie met deze larven verschilt wezenlijk van een infectie met infectieve eieren. Deze larven hoeven geen trektocht meer te maken omdat zij dit al gedaan hebben in de teef en kunnen direct in de darm volwassen worden. Op deze manier wordt de leeftijdsresistentie omzeild. Een teef die een nestje pups verzorgt gaat dus naar alle waarschijnlijkheid zelf ook weer eieren uitscheiden. Een andere verklaring voor het ontwikkelen van een patente infectie bij honden ouder dan zes maanden is dat de hond zich infecteert via predatie. Op deze manier krijgt de hond larven binnen die zich in de spieren of organen bevinden van een prooidier. Ook deze larven hoeven geen trektocht meer te maken, maar kunnen direct in de darm volwassen worden en op deze manier wordt de leeftijdsresistentie wederom omzeild en kan het tot een patente infectie komen. Er zijn echter ook minder goed te verklaren gevallen van honden ouder dan zes maanden die een patente infectie ontwikkelen.
Uit verschillende onderzoeken is echter gebleken dat het percentage honden ouder dan zes maanden dat een patente infectie doormaakt met spoelwormen minder is dan vijf tot tien procent. Dat zou betekenen dat bij minstens 90-95% van de honden op het moment van ontwormen er geen aanwijzing bestaat dat er daadwerkelijk wormen aanwezig zijn. Het advies richt zich dus op die enkele honden die wel eieren uitscheiden. Onder deze honden bevinden zich dus gevallen waarvan we niet goed weten waarom ze een patente infectie ontwikkelen. Wanneer er meer duidelijkheid zou bestaan over welke honden een patente *Toxocara* infectie ontwikkelen en waarom, zou er meer gericht ontwormd kunnen worden en minder vaak diergeneesmiddelen gebruikt hoeven te worden zonder aanwijsbare reden.

De vragen

Het doel van dit proefschrift is het verschaffen van meer duidelijkheid over de noodzaak om alle honden, ouder dan zes maanden, vier keer per jaar te behandelen zonder voorafgaande diagnose.

In dit proefschrift staan een paar vragen centraal:

- Wat is de bijdrage van de hond, ouder dan zes maanden, ten opzichte van andere diersoorten (katten en vossen) aan de contaminatie van de omgeving met spoelwormeieren?

- Welk deel van de eigenaren ontwormt regelmatig hun hond en als dit meer zou worden, wat voor invloed heeft dit op de bijdrage van de honden aan de totale contaminatie van de omgeving met spoelwormeieren?

- Wat zijn factoren die een rol spelen bij het gaan uitscheiden van spoelwormeieren voor honden ouder dan zes maanden?

- Bestaan er honden die vaker een patente infectie ontwikkelen ('wormy animals') en honden die dat bijna nooit doen? Welke factoren spelen hierbij een rol?

Een patente spoelworminfectie kan worden vastgesteld door het uitvoeren van ontlastingonderzoek. Zijn in de ontlasting spoelwormeieren aanwezig, dan wordt in het algemeen een patente spoelworminfectie als bevestigd beschouwd. Gedurende het onderzoek bleek en ook uit de literatuur blijkt dat honden nogal eens dingen eten, zoals ontlasting of prooidieren, waarin ook parasieteneieren aanwezig kunnen zijn. De via onderzoek aangetoonde eieren zijn dan niet van een infectie van de hond zelf afkomstig, maar zijn (bijna) onveranderd het maag-darmkanaal gepasseerd. Wanneer de eieren van deze parasieten die andere dieren infecteren heel anders van vorm zijn, dan is dit niet zo'n probleem omdat ze dan herkend worden als 'vreemd'. Het wordt lastiger wanneer een hond hondenpoep of kattenpoep eet, waarin spoelwormeieren zitten die nog niet infectief zijn. Dan worden via het onderzoek spoelwormeieren gevonden die niet van een eigen infectie afkomstig zijn, maar ook niet gemakkelijk hiervan te onderscheiden zijn. Een extra vraag die daarom voor het onderzoek beantwoord moest worden was dan ook:

- Hoe vaak zijn in de faeces aangetroffen spoelwormeieren het resultaat van eten van honden- of kattenpoep door de hond en in welke mate zou dit dus het onderzoek kunnen beïnvloeden?

Voor het beantwoorden van de bovenstaande vragen hebben 570 eigenaren minstens éénmaal, maar de meesten voor een langere periode, ontlasting opgestuurd van hun hond en een bijbehorende maandelijkse vragenlijst beantwoord. Dit heeft geresulteerd in 938 honden waarvan één tot wel 38 monsters zijn onderzocht.

Het onderzoek

Om een relatieve bijdrage van honden ouder dan zes maanden aan de omgevingscontaminatie met eieren van *Toxocara* te kunnen berekenen, zijn er gegevens nodig over de andere diersoorten die als eindgastheer voor deze worm hieraan ook bijdragen. Hoofdstuk 2, 3 en 4 richten zich op de bijdrage van zowel honden, vossen als katten. In **hoofdstuk 2** is gekeken naar het eerste ingezonden monster van huishonden (ouder dan zes maanden). Voor deze studie waren gegevens en uitslagen van ontlastingonderzoek beschikbaar van 916 honden. Het percentage honden dat eieren van *Toxocara* uitscheidde was 4.6%. Een aantal factoren bleek geassocieerd te zijn met het uitscheiden van eieren. De kans dat honden ouder dan twaalf maanden uitscheider waren was significant lager dan honden met een leeftijd van tussen de zes en twaalf maanden. Het percentage tijd dat de honden los mogen lopen vertoonde een duidelijke correlatie met het risico op uitscheiden van *Toxocara* eieren. Hoe hoger het percentage loslooptijd, des te groter de kans dat bij deze honden eieren in de ontlasting konden worden aangetoond. Daarnaast bleken het eten van ontlasting en recentelijk verblijf in een gastopvang (pension, kennel) ook gepaard te gaan met een grotere kans op uitscheiden van eieren. Er was geen duidelijke relatie aan te tonen tussen de ontwormingsfrequentie in de historie van de hond en de kans op het uitscheiden van eieren. In de groep honden echter die niet onlangs in een gastopvang hadden gezeten, weinig los liepen en geen ontlasting aten werden bij de honden die vier keer per jaar ontwormd werden geen patente infecties aangetoond. Uit deze resultaten valt te herleiden dat niet iedere hond een even groot risico loopt op het uitscheiden van *Toxocara* eieren en dat mogelijk hierdoor de in het verleden bij een hond toegepaste ontwormingsfrequentie weinig tot geen relatie vertoont met de kans op een actuele patente infectie bij honden.
Tevens is in **hoofdstuk 2** gekeken naar hoe de deelnemende eigenaren, voor deelname aan het onderzoek, omgingen met en aankeken tegen het ontwormen van hun hond(en) en ook met het opruimen van de ontlasting van de hond. Het grootste deel van de eigenaren vond de gezondheid van de hond de belangrijkste reden om de hond te ontwormen. Slechts 16% hield zich aan de door ESCCAP (European Scientific Counsel Companion Animal Parasites) geadviseerde gemiddelde ontwormingsfrequentie van vier keer per jaar. Hieruit blijkt dat het uitgedragen ontwormingsadvies, inclusief de reden voor ontwormen, bij de meeste deelnemers niet in de antwoorden terug te vinden is. Dit kan komen omdat men het er niet mee eens is, maar ook omdat men niet op de hoogte is. Van het in Nederland uitgedragen ontwormingsadvies, dat op geen enkele wijze verplicht is voor een eigenaar, is dus niet te verwachten dat dit tot een effectieve bestrijding leidt. Dit wordt mogelijk nog versterkt door het feit dat er geen kenniseisen worden gesteld aan de verkooppunten van ontwormingsmiddelen die overal vrij te verkrijgen zijn.

Andere diersoorten die in Nederland bij kunnen dragen aan besmetting van de omgeving met *Toxocara* eieren zijn de vossen, die dezelfde soort worm bij zich kunnen dragen en de kat, die met een eigen soort *Toxocara* besmet kan zijn. **Hoofdstuk 3** richt zich op de vossenpopulatie. Dieren die in het oosten van het land geschoten werden, zijn onderzocht op parasieten. Daar waar bij de hond ongeveer 5% van de dieren eieren van *Toxocara* bleken uit te scheiden was dit bij de onderzochte vossen maar liefst 61%. Dus, alhoewel we in Nederland waarschijnlijk veel meer honden hebben dan dat er vossen zijn, kunnen vossen, doordat ze veel vaker patente infecties hebben, toch een aanzienlijke bijdrage leveren aan de contaminatie van de omgeving. Bij de onderzochte huiskatten is het verschil minder duidelijk. Zoals in **hoofdstuk 4** te lezen is, scheidt ongeveer 7% van de huiskatten eieren uit van *Toxocara.* Hierbij moet wel in gedachten worden gehouden, dat de onderzochte katten vooral katten waren die de behoefte doen op de kattenbak. Dit zou een vertekend beeld kunnen geven van de gemiddelde huiskat in Nederland en tevens zijn geen zwerfkatten meegenomen in het onderzoek. Waarschijnlijk is die 7% dus een onderschatting van de werkelijke bijdrage van katten aan de omgevingsbesmetting.
Omdat alle genoemde percentages van *Toxocara* eieren uitscheidende dieren gebaseerd zijn op microscopisch onderzoek van de ontlasting en het aantonen van eieren, is in **hoofdstuk 5** onderzocht in welke mate het eten van ontlasting door honden hierop verstorend kan werken. Coprofagie, zoals het eten van ontlasting wordt genoemd, is een veelvuldig bij honden voorkomend gedrag. Bij katten komt dit gedrag bijna niet voor en bij vossen is het niet bekend hoe vaak dit voor komt. Wanneer een ontlastingmonster van honden eieren bevatte, dan zou dit dus kunnen komen door een echte infectie, of doordat de eieren zijn opgegeten en het maag-darmkanaal onveranderd zijn gepasseerd, vergelijkbaar met bijvoorbeeld een maiskorrel. Bijna de helft van de deelnemende eigenaren herkent coprofagie bij de eigen hond(en). Vanwege deze mogelijke verstoring van het onderzoek is bij een ontlastingmonster dat positief testte op eieren aan een eigenaar een nieuw monster gevraagd dat genomen werd nadat een hond drie dagen lang geen ontlasting heeft kunnen eten. Wanneer dit herhalingsmonster ook positief testte op dezelfde soort parasieteneieren, dan werd de infectie als 'bevestigd' beschouwd en anders als 'negatief'. Van de *Toxocara* positieve ontlastingmonsters werd 49% van de herhalingsmonsters negatief getest en werden er dus geen eieren terug gevonden. Daarom is voor de analyses in alle onderzoeken coprofagie bij de hond steeds als factor meegenomen.
De bijdrage van de honden aan de omgevingsbesmetting met *Toxocara* eieren in Nederland, ten opzichte van die van huiskat, zwerfkat en vos, met de focus op dieren die ouder zijn dan een half jaar, is geschat in **hoofdstuk 6**. Gegevens van eerdere studies en vanuit de literatuur zijn gebruikt als input voor een nieuw model ter verfijning van een eerder beschreven model. Uit dit nieuwe model bleek de hond over het algemeen in Nederland de grootste bijdrage (39%) te leveren aan de omge-

vingsbesmetting met *Toxocara* eieren. Deze positie ging echter verloren wanneer de gezelschapskatten en zwerfkatten als één groep worden beschouwd. De kat is dan verantwoordelijk voor 46% van de eieren in de omgeving. De diersoort die voor het grootste deel bijdraagt aan de omgevingsbesmetting kan verschillen met de graad van verstedelijking van een gebied. Met dit model kon ook gesimuleerd worden wat de invloed is van verschillende percentages eigenaren die ontwormen in verschillende frequenties. Hetzelfde was mogelijk voor het opruimen van ontlasting. Hieruit bleek dat wanneer 90% van de eigenaren die op dit moment niet vier keer per jaar ontwormt dit wel zou doen, dat dan de geschatte bijdrage van de huishond van 39% daalt tot 28%. Voor een meer aanzienlijke daling in de bijdrage zal dus het grootste deel van de hondeneigenaren vaker dan vier keer moeten gaan ontwormen. Dit lijkt op dit moment niet realistisch. Het opruimen van de ontlasting van hun hond door de eigenaar heeft een vergelijkbaar effect met maandelijks ontwormen. Onder de huidige omstandigheden is opruimen van de ontlasting beter in een beleid op te nemen en het is bovendien makkelijker te controleren dan ontwormen op basis van vrijwilligheid.

Leveren alle honden een vergelijkbare bijdrage aan de contaminatie van de omgeving of maken sommige honden vaker een patente infectie door dan andere? Deze vraag is behandeld in **hoofdstuk 7**. Hiervoor zijn alle ontlastingmonsters en antwoorden op de enquêtes die beschikbaar waren, meegenomen en geanalyseerd. Bij het grootste gedeelte van de groep deelnemende honden (67,9%) kon gedurende de maanden van het onderzoek geen patente infectie worden aangetoond. Bij de andere honden zijn in totaal 585 ontlastingmonsters positief getest op eieren van *Toxocara*. Van deze positieve monsters waren er 421 afkomstig van honden waarbij meer dan één keer een patente infectie is aangetoond. Deze groep honden (14,6% van de groep deelnemende honden), die meerdere malen een patente infectie door heeft gemaakt, was dus verantwoordelijk voor 72% van de positieve monsters. Het opsporen van deze honden die herhaaldelijk patente infecties doormaken en deze honden vervolgens frequenter behandelen, zou dus een effectievere aanpak kunnen zijn dan alle dieren blind vier keer per jaar behandelen.

Het lijkt erop dat het herhaaldelijk doormaken van een patente infectie gekoppeld kan worden aan risicofactoren die mogelijk invloed hebben op de afweer van een dier en daardoor op reactivatie van larven die al ergens in het lichaam in ruste waren gegaan. De terugkerende infecties waren geassocieerd met bijvoorbeeld het toedienen van corticosteroïden, veranderingen in het levenspatroon van de hond en eigenaren die regelmatig een dierenartsenpraktijk bezoeken. Het sporadisch doormaken van een patente infectie, daarentegen, lijkt onder andere samen te hangen met bijvoorbeeld het opeten van dingen uit de omgeving (inclusief ontlasting), percentage loslooptijd en dieetinvloeden.

Opvallend was ook dat er een seizoensmatige variatie in het voorkomen van patente

infecties zichtbaar was gedurende de studie met een jaarlijkse piek in de winter. Alhoewel dit niet volledig verklaard kon worden, wijst dit wel uit dat het effect van het blind ontwormen per seizoen kan verschillen.

Conclusies en vooruitzichten voor de toekomst

Honden zijn voor een belangrijk deel verantwoordelijk voor de omgevingscontaminatie met eieren van *Toxocara*. Eigenaren moeten zich ervan bewust zijn dat dit een risico met zich mee brengt voor de volksgezondheid. Het grootste deel van de deelnemende eigenaren herkende de volksgezondheid echter niet als de belangrijkste reden voor het ontwormen van hun hond. Slechts een beperkt aantal eigenaren ontwormt hun hond(en) volgens de geadviseerde vier keer per jaar. Het regelmatig opruimen van ontlasting van de honden wordt ook maar door een beperkt deel van de deelnemende eigenaren uitgevoerd. Een belangrijke conclusie is ook, dat zowel katten als vossen eveneens een grote bijdrage leveren aan de totale besmetting van de omgeving met *Toxocara* eieren. Indien het gewenst is dat de bijdrage door honden volledig wordt teruggedrongen vanwege een zoönotisch risico voor de mens, zou dit dus gepaard moeten gaan met gelijksoortige bestrijding van spoelworminfecties bij katten en vossen.

Niet alle honden lijken een even groot risico te lopen op het doormaken van een patente infectie. Het lijkt voor een groot deel van de onderzochte honden niet noodzakelijk om hen vier keer per jaar blind te ontwormen. Het regelmatig uitvoeren van diagnostiek kan helpen om juist die honden op te sporen die vaker een patente infectie doormaken dan anderen. De frequentie echter waarin deze diagnostiek in het begin uitgevoerd dient te worden om een goed beeld te krijgen, is waarschijnlijk hoog en vrijwillige medewerking van eigenaren hieraan valt te betwijfelen vanwege hogere kosten. Onderzoek is nodig naar hoe eigenaren kunnen worden gemotiveerd om mee te werken aan regelmatige diagnostiek, alsmede om faeces van de eigen hond beter op te ruimen. Ontwormen op maat vindt al plaats, bijvoorbeeld bij honden die jonger zijn dan een half jaar. Ook de beslisboom van ESCCAP stuurt aan op een advies dat meer op maat is gemaakt. Bovendien bieden de risicofactoren genoemd in de hoofdstukken 2 en 7 handvatten om honden meer op maat te behandelen.

ENGLISH SUMMARY

Background

Toxocara canis, the roundworm of the dog, is the most common parasitic worm in dogs in the Netherlands. In adult dogs this worm usually causes little or no symptoms. However, the worm can cause serious illness in puppies. Also in humans syndromes are known which are attributable to wandering larvae in the body. *Toxocara* is therefore considered a zoonotic parasite. Because of its ability to cause disease in young animals and in particular also because of its zoonotic potential, *Toxocara* is the main reason and target for the current propagated deworming advice in the Netherlands. Dogs up to the age of six months are frequently treated, varying from deworming them once every two weeks to deworming them on monthly basis. This is a higher frequency than is advised for older animals. There is no debate concerning the deworming advice for these young dogs. They are already infected before birth in the uterus and after birth by ingesting milk that contains larvae of *Toxocara*. All of these mentioned routes of infection may result in a so-called patent infection in a puppy, meaning that a puppy will shed large numbers of eggs with the faeces. In fresh stool, these eggs are not immediately infective. They first need to develop a few weeks in the environment before a larva appears in the eggs and the eggs become infective. After ingestion by a dog, these infective eggs can lead to new infections. The larvae that hatch from the eggs, need to migrate through the body and will enter the lungs. After being coughed up and swallowed, the larvae become adults in the intestines of a dog and can cause another patent infection. Somewhere between three and six months of age, dogs are believed to develop a so-called “age resistance”. This means, when a dog older than six months gets infected via the ingestion of infective eggs, this usually does not lead to adult worms in the gastrointestinal tract of the dog. The infection will remain limited to somewhere in the body where the larvae become dormant. Therefore, most dogs older than six months will not develop patent infections. Hence, it is questioned by owners and veterinarians whether all dogs older than six months need to be dewormed at identical intervals.
When infective eggs are ingested by other animal species, including humans, this will not lead to adult worms in the intestines. But like in older dogs, the larvae that have hatched from the eggs will start their migration, only to get stuck somewhere in the body and become dormant. Depending on the place where such a larva eventually ends up, this may lead to symptoms. Because humans can be infected and this can sometimes lead to complications the general advice is to deworm each dog older than six months at least four times a year. Despite the fact that ‘age resistance’ is supposed to be effective in dogs older than six months, it is known that some dogs do develop a patent infection. Some of these cases can be explained, for example in nursing

bitches. Here, the reactivated larvae start to migrate through the body. This way they can infect puppies in the uterus and after birth also through the mammary glands while the new born puppies are drinking. When the nursing bitch performs litter care and ingests the faeces of the puppies, she can ingest larvae that have passed and survived the gastro-intestinal tract of the puppies and develop a patent infection herself. An infection of a dog older than six months with larvae differs substantially from an infection with infective eggs. Ingested larvae do not tend to migrate through the body because they already done this in the bitch (infection by infective eggs and migration to the mammary glands) and they are ready to mature in the intestine without further migration. This way the 'age resistance' is circumvented. Therefore, a bitch that is performing litter care will most likely develop a patent infection herself. Another explanation for the development of a patent infection in dogs older than six months is when a dog gets infected by consuming a prey animal. In this way, the dog ingests larvae which are located in the muscle tissue or organs of an animal prey. These larvae also do not need to migrate through the dog's body anymore, but can develop into adult worms directly in the intestine. Again, 'age resistance' will be circumvented leading to a patent infection. However, there are cases of dogs older than six months that develop a patent infection which cannot be explained by these two scenario's.

Various studies have shown that the percentage of household dogs, older than six months, that actually develop a patent *Toxocara* infection is less than five to ten percent. That would mean that, when blind deworming is practiced, at least 90-95% of the dogs will be dewormed, at any given moment, without indication that there actually are adult worms present in the intestines. The current general advice to deworm all dogs regularly is therefore solely based on those few dogs that actually shed eggs. If we could predict what situations are associated with higher chances of developing patent infection in these dogs, treatment advice might become more focused accompanied by a less frequent use of veterinary drugs without any (diagnostic) evidence.

The questions

The aim of this thesis is to provide some clarification about the need to treat all dogs older than six months, four times a year without the use of diagnostics.
Key questions addressed in this thesis are:

- What is the contribution of dogs older than six months, compared to other animals (cats and foxes) to the contamination of the environment with *Toxocara* eggs?

- What is the attitude of participating owners towards regular deworming

of their dog(s)? When more owners would practice blind deworming, how would this affect the relative contribution of dogs to the overall contamination of the environment with roundworm eggs?

- What factors appear to be associated with the occurrence of patent *Toxocara* infections in dogs older than six months?

- Are all dogs equally at risk for developing a patent infection or do some dogs more frequently develop a patent infection than others (wormy animals)? And if so, what factors are associated with such recurrent patent infections?

Patent roundworm infections can be determined by performing coproscopical examination of fecal samples. When roundworm eggs are present in the faeces it is usually considered as a patent roundworm infection. However, literature shows that it is not uncommon for dogs to eat things such as faeces from other animals or preys, which may contain parasite eggs. Clearly, such ingested eggs do not originate from an actual infection of the dog itself, but just pass the gastro-intestinal tract. When these passing eggs are morphologically very different this does not pose a problem. However, interpretation of results from coproscopical examination of faeces becomes much more problematic when a dog eats faeces from another dog or cat containing roundworm eggs. Those eggs are usually indistinguishable from those *Toxocara* eggs that result from an actual infection of the dog itself. Therefore, passing roundworm eggs will lead to a false-positive diagnosis of a *Toxocara* infection. Consequently, an additional question in need of an answer for a proper interpretation of the results was:

- How often are roundworm eggs in the faeces of a dog the result of eating faeces from a dog or cat with a patent infection, and to what extent did this influence the results in the present study?

To answer the questions mentioned above, 570 owners submitted a faecal sample of their dog(s) and answered a monthly questionnaire. This resulted in 938 dogs from which one up to 38 faecal samples were investigated and corresponding questionnaires were analyzed.

The results

To calculate the relative contribution to the environmental contamination with *Toxocara* eggs of dogs older than six months, data are required from other potential definitive hosts for this roundworm . Chapters 2, 3 and 4 focus on the contribution

of dogs as well as foxes and cats. **Chapter 2** deals with the first faecal sample and questionnaire that was submitted from household dogs (over six months old). For this study, data and coproscopical results were available from 916 dogs. The percentage of dogs shedding *Toxocara* eggs was 4.6%. Several factors appeared to be associated with the shedding of eggs. The probability that dogs older than twelve months were shedding was significantly lower than dogs in the age category of six to twelve months. The percentage of time that dogs were allowed to walk off-leash showed a clear association with the risk of shedding *Toxocara* eggs. Eating faeces from other animals and recent stay in a kennel or pet hotel were also associated with a higher probability of shedding eggs. No clear relationship could be detected between how frequent dogs were dewormed before they participated in the study and the probability of excreting *Toxocara* eggs. However, in the group of dogs, which were not recently being kenneled, had relatively little walking time off-leash, and that did not eat faeces from other animals a deworming frequency of four times a year appeared to be slightly associated with the absence of patent infections.
From these results it can be concluded, that not every dog shares the same risk of shedding *Toxocara* eggs. This may partly explain the lack of association between the applied deworming frequency and the probability of having a patent infection.
How the participating owners dealt with and felt about deworming their dog(s) and cleaning up the faeces of their dog(s) is also addressed in **chapter 2**. Most of the owners mentioned that the dog's health is the main reason for deworming their dog. Only 16% of the participating owners followed the advice given by ESCCAP (European Scientific Counsel Companion Animal Parasites) to deworm four times a year on average. The disseminated deworming advice, including the main reason for this deworming advice, is not reflected in the answers of most participants. Whether participating owners did not agree with it or were not aware of it was not clear. Effective *Toxocara* control cannot, under the given circumstances, be expected from current recommendations, which are not mandatory for dog owners. Moreover, there is no defined minimum level of knowledge required for retail points that sell anthelmintics over the counter without any veterinary involvement.
Among the other definitive host species are foxes and cats. Foxes can have a patent infection with the same species of roundworm as dogs. Cats, however, have their own species of roundworms, which is also zoonotic. So, both foxes and cats contribute to the overall contamination of the environment with *Toxocara* eggs in the Netherlands. **Chapter 3** focuses on the fox population. Foxes that were killed in the east of the country, were examined for parasites. Where the prevalence of egg shedding in dogs was almost 5%, in foxes no less than 61% were found to shed these roundworm eggs. So, although the number of dogs in the Netherlands exceeds the number of foxes by far, foxes can because of the high prevalence still contribute significantly to the contamination of the environment with *Toxocara* eggs. In household

cats that were studied in **chapter 4**, about 7% shed *Toxocara* eggs. It should be borne in mind that the studied population of cats were all cats that used the litter box. This could have biased the outcomes. Because of logistic reasons concerning the difficulty of collecting samples and obtaining information, stray cats were not included in the study. Therefore, a prevalence of 7% probably will lead to an underestimation of the actual contribution of cats to the environmental contamination.
All the percentages of *Toxocara* egg shedding animals as mentioned above are based on the presence of eggs after microscopic examination of faecal samples. In **chapter 5** the question is raised to what extent eating faeces by dogs could influence the outcome of this diagnostic method. Coprophagy, as eating faeces is called, is common in dogs, uncommon in cats and for foxes information is lacking. When a faecal sample tested positive for parasite eggs, this could be explained either by a true patent infection or by eggs that were ingested by a dog and apparently passed the gastro-intestinal tract unaltered. Almost half of the participating owners recognized coprophagic behavior in their own dog(s). Interference with the outcomes of our diagnostic procedure was therefore to be expected. When a faecal sample tested positive for parasite eggs, the owner was asked for a new sample that was taken after a period of three days in which the owner prevented the dog from eating things from the ground. If this confirmation sample also tested positive for the same type of parasite eggs, an infection was considered “confirmed”. However, if the eggs were not present in the confirmation sample, or this sample contained different types of eggs, it was considered “negative”. Of the *Toxocara* positive stool samples, 49% of the confirmation samples tested negative, meaning that no *Toxocara* eggs could be diagnosed. Therefore, in the analyses of further results of the studies coprophagy was always included as an important factor in dogs.
The relative contribution of dogs to the environmental contamination with *Toxocara* eggs in the Netherlands, compared to that of domestic cats, stray cats and foxes, is estimated in **chapter 6**. Data from previous studies and from literature were used as input for a new model to refine a previously described model. Our new model indicated that the dog indeed is contributing most (39%) to the environmental contamination with *Toxocara* eggs in the Netherlands. However, this position was lost when the household cats and stray cats were considered as one group. In this case, the cat appears to be responsible for 46% of the eggs in the environment. Depending on the degree of urbanization of an area it can differ to which extent an animal species is responsible for the major part of contamination of the environment. This model could also be used to simulate the effect of different percentages of owners deworming their dogs at different frequencies on the relative contribution of household dogs. The same thing was possible for the compliance of owners to cleaning up faeces after their dog. This showed that if 90% of the dog owners that do not deworm their dogs four times a year would actually do so, the contribution of the household dogs

would drop from 39% to only 28%. For a more substantial decrease in the relative contribution the majority of dog owners need to deworm their dogs more often than four times per year. This does not seem to be realistic at present times. Cleaning up the faeces of dogs by their owners has a similar effect as deworming monthly. Under the current circumstances, with anthelmintics for dogs being freely available, cleaning up faeces probably fits more easily into a *Toxocara* control policy because it is more easy to check than deworming dogs on a voluntary basis.
Do all dogs, older than six months of age, contribute equally to the environmental contamination or do some dogs appear to have patent infections more frequently than others? This question is addressed in **chapter 7**. All available faecal samples (n=12,968) and answers to the questionnaires were analyzed for this purpose. The majority of the dogs (67.9%) did not show a patent infection during the period they participated in this study. From the other dogs a total of 585 faecal samples tested positive for *Toxocara* eggs. Of these, 421 samples came from dogs with more than one patent infection during the study. This group of frequently shedding dogs (14.6% of the group of participating dogs) was responsible for 72% of the *Toxocara* positive samples. By identifying these dogs, that show recurrent patent *Toxocara* infections and by treating these dogs more frequently, a greater efficacy can be expected compared to treating all animals blindly four times a year.
It seems likely that the recurrent patent infections are somehow associated with risk factors impacting the functionality of the immune response of a dog. When the immune system is somehow compromised, larvae already present in a dog's body may become reactivated. Indeed, recurrent infections appeared to be associated with, for example, the administration of corticosteroids, changes in the lifestyle/function of a dog, and a proxy of owners visiting a veterinary practice on a regular basis. The more sporadically occurring patent infections, by contrast, seem to be associated among other things to eating stuff from the environment (including faeces), percentage of time walking off-leash and dietary influences. Finally, a remarkable seasonal pattern in the incidence of patent infections was observed during the study, with an annual peak in wintertime. Although this could not be fully explained, it indicates that the effect of a blind deworming strategy may vary by season.

Conclusions and prospects for the future

Dogs are largely responsible for the environmental contamination with *Toxocara* eggs. Owners should be aware that this can compromise public health. The majority of the participating owners, however, did not recognize public health as the main reason for deworming their dog. Only a limited number of owners dewormed their dog(s) according to the recommended four times a year. And only a small group of the participating owners acknowledged to clean up faeces from their dogs on a

regular basis. An important conclusion is that both cats and foxes are responsible for a considerable part of the contribution to the overall contamination of the environment with *Toxocara* eggs. Strategies to control disease due to *Toxocara* infections in humans must therefore also aim for controlling this roundworm in (stray) cats and foxes.

Not all dogs appear to be equally at risk for developing patent *Toxocara* infections. For the majority of the participating dogs it did not appear to be necessary to get dewormed four times a year. Regular faecal examination could help to identify dogs showing recurrent patent infections. The frequency, however, in which coproscopical examination should be performed is likely to be high and voluntary cooperation of owners to do so is not expected because of higher costs. There is a need for studies how to improve the involvement of owners in programs based on performing regular coproscopical examination, as well as in better cleaning up faeces from their own dog(s). Targeted deworming is already advocated, for example for dogs younger than six months or in lactating bitches. ESCCAP also created a crude decision tree for a more customized deworming. The risk factors listed in **Chapters 2** and **7** can be used to further refine such decision trees, both in terms of blind anthelmintic treatment as in creating customized preventive health care including coproscopical monitoring, which should lead to a substantial reduction in unnecessary use of medicine.

DANKWOORD

Mijn naam staat dan wel op de kaft van dit proefschrift, maar het was nooit zover gekomen zonder de inspiratie, motivatie en hulp van velen. Dus hierbij wil ik graag *iedereen bedanken* die zich hierin herkent.
Echter, een deel zal nu waarschijnlijk denken: ja lekker dan, ik heb wel meer gedaan dan alleen wat inspireren, motiveren en helpen. Ik was toch echt wel een hele steun. Voor deze mensen geldt dat ik hen ook zeker *heel erg* wil *bedanken*.
Nu zal er nog steeds een groep zijn die het gevoel heeft dat met zo'n algemeen dankwoord geen eer wordt gedaan aan hun bijdrage omdat zij mij bijvoorbeeld op de wereld hebben gezet, het toen en daarna dagelijks met mij uit hebben weten te houden. Of zij die juist die noodzakelijke, vakinhoudelijke of sociale bijdrage hebben geleverd die tot een mooi einde van deze promotie heeft geleid. Voor hen wil ik graag ***mijn enorme dank*** vermelden.
In ieder geval, allen bedankt voor de reis naar hier en nu…… op naar de volgende uitdaging.

…… maar natuurlijk:
Lieve Christine, dank. Dat hebben we dan toch maar weer mooi gedaan!

Cartoon op ommezijde:
Naar een cartoon van Bill Watterson © 1995

CURRICULUM VITAE

Rolf Nijsse was born in Middelburg on the 14th of December in 1969. He went to primary school at the 'van Duyvenvoordeschool' in Oost Souburg and obtained his VWO diploma in 1988 at 'de Stedelijke ScholenGemeenschap Middelburg'. In that same year he started studying veterinary sciences at Utrecht University where he graduated in 1996. After working in several veterinary clinics in the Netherlands he started working at 'het Groenhorstcollege' in Barneveld. After seven years of teaching at this school for veterinary technicians he started as a teacher at the Faculty of Veterinary Medicine at Utrecht University in 2004. Here he combines teaching veterinary students with research on companion animal parasites and supporting the Veterinary Microbiological Diagnostic Centre. As secretary for the European Scientific Counsel Companion Animal Parasites (ESCCAP) Benelux he is actively involved in constructing a sound advice for controlling parasitic infections in companion animals.