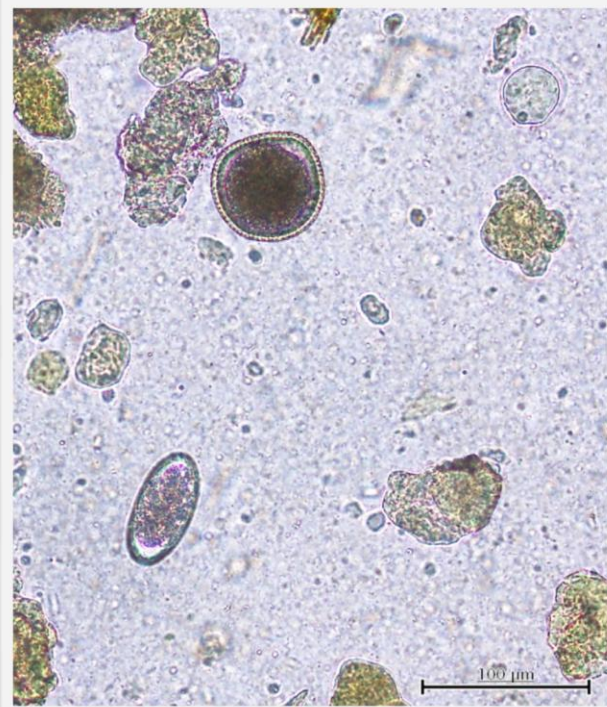


Research Project Veterinary Medicine

The prevalence of household dogs shedding helminth eggs and/or (oo)cysts in the Netherlands and the association with reported coprophagy



<http://www.vetwest.com.au/pet-library/coprophagia-in-dogs-stool-eating>



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Abstract

Certain helminth eggs and oocysts excreted in a dog's faeces may originate from intestinal passage after coprophagia. As a consequence, the results of faecal examination can be false positive for patent infections. The objective of this study was to investigate to what extent coprophagia interferes with the prevalence of dogs shedding helminth eggs and/or (oo)cysts. As part of a longitudinal study, dog owners with a dog older than 6 months, submitted a faecal sample monthly and filled out a questionnaire. A total of 5711 faecal samples were analyzed during my research period (February 3, 2014 until April 4, 2014) and during the period March 1, 2012 to March 1, 2013. The faecal samples were examined for the presence of intestinal parasites using a Centrifuge Sedimentation Flotation technique with sucrose solution as flotation medium (density: 1,27-1,30 g/cm³). If a sample tested positive for *Toxocara* spp., *T. vulpis*, canine hookworms and/or *Capillaria* spp. at the first examination, the owner was asked to submit a second faecal sample (also referred as confirmation sample) after keeping the dog from eating faeces for three days. In this way, differentiation between passive passage and patent infection was possible.

A remarkable percentage of positive samples returned as negative confirmation samples: 40,9% for *Toxocara* spp. eggs, 69,6% for strongyle-type eggs and 53,8% for *T. vulpis* eggs between March 2012 and March 2013. During my research period in 2014, 40% of the confirmation samples for *Toxocara* spp. eggs and 100% of the confirmation samples for strongyle-type eggs were negative. Contrary to our expectations, no significant association was found between reported coprophagy and the number of negative confirmation samples. The finding of non-dog typical parasites in the faeces of not coprophagic dogs, proved that the owners' perception of the coprophagic behaviour of their dog is not always reliable. In conclusion, results showed that a considerable percentage of infections with *T. canis*, *T. vulpis* and hookworms diagnosed by a single faecal examination can be false positive. In dogs, prevalence estimates for patent helminth infections are usually based on a single faecal examination. Prevalence rates reported in cross-sectional prevalence studies may thus be overestimated and require cautious interpretation. Repeated faecal examinations are recommended to confirm patent infections. Further studies with a higher compliance of owners providing confirmation samples are necessary for a reliable determination of the exact influence of coprophagia on the prevalence of household dogs shedding helminth eggs and (oo)cysts.

1. Introduction

In this study we investigated the association between reported coprophagia and the prevalence of household dogs shedding helminth eggs and/or (oo)cysts in the Netherlands.

1.1 Coprophagial behavior

Consumption of faeces, or coprophagia, is not uncommon among dogs (Fahrion et al., 2011; Overgaauw & van Knapen, 2013). However, research about coprophagia in dogs is sparse (Boze, 2008). Little is known about the prevalence of coprophagia in the dog population in the Netherlands (van der Borg & Graat, 2006).

In dogs eating faeces, two main categories can be distinguished: consumption of dog faeces (intraspecific coprophagia) and consumption of faeces from other species, such as cats, horses, rabbits and humans (interspecific coprophagia) (van der Borg & Graat, 2006). Intraspecific coprophagia can be further divided into two types: consumption of own faeces (autocoprophagia) and consumption of faeces from another dog (allocoprophagia) (Boze, 2008; van der Borg & Graat, 2006). Many dog owners consider coprophagia as an undesirable behavior (Boze, 2008). However, in some situations coprophagia is a normal behavior for canines, like the care of a bitch toward her litter (Boze, 2008; Landsberg & Denenberg, 2012). Bitches consume the faeces and urine of their puppies to keep the litter clean and to prevent detection by predators (Boze, 2008). Coprophagia in puppies can also be considered as a normal behavior for a short period (Beaver, 2009). It is likely to be part of exploratory behavior and may be useful to establish normal flora in the intestinal tract (Beaver, 2009; Landsberg & Denenberg, 2012). Coprophagia outside of these situations can be considered as abnormal (van der Borg & Graat, 2006).

The etiology of coprophagia in dogs is not fully understood, however in the literature several hypotheses have been mentioned (van der Borg & Graat, 2006; Wells, 2003). For example, coprophagia may be caused by medical disorders like exocrine pancreatic insufficiency and malabsorption (Beaver, 2009; Landsberg & Denenberg, 2012). It is important to rule out medical causes before starting a treatment (Beaver, 2009; Boze, 2008). Nutritional problems can also play a role, but this is unlikely in dogs eating commercial pet foods (van der Borg & Graat, 2006). Another hypothesis is that coprophagia may be caused by boredom and/or stress (Boze, 2008; van der Borg & Graat, 2006). The behavior seems to be more common in dogs kept in a barren environment and in dogs who do not get enough exercise (Beaver, 2009). Furthermore, it has been suggested that the attention dogs receive from their owners when they are eating faeces may reinforce the behavior as a result of operant conditioning (Boze, 2008; Wells, 2003). The taste of faeces may also contribute to the behavior, because if it is appealing to the dog it may act as a positive reinforce (Wells, 2003). It is difficult to start a successful treatment if the cause of the behavior is still unclear (van der Borg & Graat, 2006).

1.2 The ingestion of helminth eggs and/or (oo)cysts by coprophagia

Considering that the faeces from another animal may include helminth eggs and/or (oo)cysts, coprophagia in dogs may result in the dog being infected. Therefore, the dog has to be a suitable definitive host of the parasite and the dog needs to ingest the infective stage of an egg or (oo)cyst. Sometimes the eggs or (oo)cysts are not immediately infective after shedding, because they need some time in the environment to develop to the infective stage. If this

development takes several weeks or months, the chance of a dog becoming infected after coprophagia is very small, because in the meantime it is likely that the faecal material already has been dispersed. Table 1 shows the average time necessary to develop to the infective stage of the for this study relevant helminths and protozoa of dogs, including *Toxocara canis*, *Trichuris vulpis*, hookworms (*Uncinaria stenocephala* and *Ancylostoma caninum*), *Capillaria* spp., *Taenia* spp., *Echinococcus* spp., *Cystoisospora* spp. (also called *Isospora* spp.) and *Giardia duodenalis* (Claerebout, 2008; Companion Animal Parasite Council, 2012b; Companion Animal Parasite Council, 2013a; Companion Animal Parasite Council, 2013b; Overgaauw & van Knapen, 2013; Taylor et al., 2007; Traversa, 2011).

Table 1 Mean time necessary to develop to the infective stage

Species	Excreted structure	Eggs or (oo)cysts excreted in faeces	Infective stage	Mean time necessary to become infective***
<i>Toxocara</i> spp.	Egg	Non-infective	Egg containing L3	3 weeks to several months
<i>Trichuris vulpis</i>	Egg	Non-infective	Egg containing L1	3 to 8 weeks
Hookworms*	Egg	Non-infective	Free-living L3	2 to 9 days
<i>Capillaria</i> spp.	Egg	Non-infective	Egg containing L1	5 to 6 weeks
Taeniids**	Egg	Infective	Egg containing a hexacanth embryo	-
<i>Cystoisospora</i> spp.	Oocyst	Non-infective	Sporulated oocyst with 2 sporocysts	< 16 hours****
<i>Giardia duodenalis</i>	Cyst	Infective	Cyst	-

* Including *Uncinaria stenocephala* and *Ancylostoma caninum*

** Including *Taenia* spp. and *Echinococcus* spp.

*** The time necessary to develop to the infective stage depends on climate and soil type

**** Under favorable conditions (temperatures between 30°C and 37°C) sporulation occurs in less than 16 hours

Besides the dog becoming infected, coprophagia may result in intestinal passage of helminth eggs and/or (oo)cysts and subsequently the secretion of these eggs or (oo)cysts in the dog's faeces while the dog is not infected. To achieve a false positive diagnosis for patent infection, the stages must still be recognizable as eggs or (oo)cysts of the parasites after passing the gastrointestinal tract of a dog. In the literature we have searched for evidence whether the eggs of *Toxocara* spp., *Trichuris* spp., strongyle type (*U. stenocephala* and *A. caninum*), *Capillaria* spp., *Taenia* spp. and *Echinococcus* spp. and the (oo)cysts of the protozoan parasites *Cystoisospora* spp. and *G. duodenalis* are able to remain recognizable while passing the digestive tract of a dog. To our knowledge, there is practically no evidence based literature about this topic. Therefore we have made assumptions based on the findings in our laboratory, the life cycles of the parasites and the characteristics of the eggs and (oo)cysts.

1.3 Capability of passing the gastrointestinal tract of a dog

As mentioned before, we want to know whether the eggs and (oo)cysts of the for this study relevant helminths and protozoa can pass the gastrointestinal tract of a dog and still can be recognized as egg or (oo)cyst. This study was part of a longitudinal study which started in 2012. Participating dog owners were asked to send a faecal sample of their dog monthly to the laboratory. We were able to make assumptions based on the findings in our laboratory of the last two years and the characteristics of the intestinal parasites.

Toxocara spp. eggs have a thick, pitted shell and are resistant to common disinfectants (Companion Animal Parasite Council, 2012a; Taylor et al., 2007). Dogs and foxes are the definitive hosts of *Toxocara canis* and cats are the definitive hosts of *Toxocara cati* (Taylor et al., 2007). Fahrion et al. (2011) reported that following ingestion of felid faeces, dogs may shed *T. cati* eggs in their faeces after intestinal passage. *Toxocara* spp. eggs can thus be considered capable in passing the digestive tract of a dog without being morphologically affected. Our lab results showed *Toxocara* spp. eggs of different sizes. *T. canis* eggs may be slightly larger than *T. cati* eggs, however some studies concluded that egg measurement is an insufficient criterion to differentiate *Toxocara* spp. eggs (Uga et al., 2000). The study of Uga et al. (2000) showed that approximately 90% of the *T. cati* and *T. canis* eggs measured are of similar size. A PCR technique can be used to differentiate between *Toxocara* spp. However, a PCR is more expensive, requires more time than microscopy and does not differentiate between dogs with a patent *T. canis* infection and dogs that shed *T. canis* eggs due to the consumption of faeces from another dog or fox (Fahrion et al., 2011; Nijse et al., 2014). Fahrion et al. (2011) suggested to take egg measurement into account as a first indicator in routine diagnostics. In conclusion, we consider *Toxocara* spp. eggs as able to remain recognizable while passing the digestive tract of a dog. The finding of *Toxocara* spp. eggs by faecal examination thus does not necessarily mean that the dog is infected with *T. canis*.

Trichuris vulpis eggs have a thick smooth shell and are very resistant to environmental conditions (Taylor et al., 2007). The embryonated eggs may survive in the environment for several years. In agreement with R. Nijse (personal communication, March 12, 2014), we consider *T. vulpis* eggs as able to pass the gastrointestinal tract of a dog. *Trichuris* spp. eggs may thus be found in a faecal sample due to intestinal passage after coprophagia. This assumption is partially based on the finding of *Trichuris* spp. eggs that were too small to be from *T. vulpis* in dog's faeces examined in our laboratory.

Canine hookworms (like *Uncinaria stenocephala* and *Ancylostoma caninum*) produce strongyle type of eggs. Strongyle-type eggs have a thin wall and therefore we consider the eggs as not likely to be very resistant to the conditions in the dog's digestive tract. However according to R. Nijse (personal communication, March 12, 2014), unpublished data proved that strongyle-type eggs are able to pass the gastrointestinal tract of a dog seemingly unaffected. Our lab results support this conclusion; we found frequently strongyle-type eggs that were too large or too small to be from *U. stenocephala* (71-93 x 35-58 μm) and *A. caninum* (56-75 x 34-47 μm) (Companion Animal Parasite Council, 2012b; Taylor et al., 2007). In conclusion, strongyle-type eggs may be found in the dog's faeces while the dog is not infected, due to passive passage of eggs.

Capillaria spp. eggs (like *Capillaria aerophila* and *Capillaria boehmi*) resemble those of *Trichuris* spp. in having a thick shell and bipolar plugs (Taylor et al., 2007). The life cycles of these *Capillaria* spp. are not completely understood, but it is suggested that an egg containing a first-stage larva is the infective stage (Claerebout, 2008; Conboy, 2009b). *Capillaria* spp. eggs can survive in the environment for months (Taylor et al., 2007). We expect *Capillaria* spp. eggs as being able to remain recognizable while passing the digestive tract of a dog. Our lab results support this assumption, because we found *Capillaria* spp. eggs in dog's faeces which did not match the size of *C. aerophila* (59-80 x 30-40 μm) or *C. boehmi* (54-60 x 30-35 μm) (Conboy, 2009b; Taylor et al., 2007). It is thus considered possible to find *Capillaria* spp. eggs in a faecal sample of a dog due to coprophagia.

Dogs serve as definitive hosts of different *Taenia* species and *Echinococcus* species and become infected by ingesting larval cysts developed in infected intermediate hosts (Companion Animal Parasite Council, 2013b; Taylor et al., 2007). Dogs with a tapeworm infection excrete proglottids containing eggs in their faeces (Conboy, 2009a). The eggs are immediately infective to the intermediate host. The infective eggs contain a hexacanth embryo (also known as oncosphere), have a thick wall and remain viable for many months or up to one year in a moist environment at low temperatures (Conboy, 2009a; Thompson & McManus, 2001). Suitable intermediate hosts are infected after oral intake of tapeworm eggs (Taylor et al., 2007). Once *Echinococcus* spp. eggs reach the stomach of the intermediate host, the oncosphere will be released in response to enzymes of the stomach and small intestine (Thompson & McManus, 2001). Subsequently, the oncosphere penetrates through the wall of the small intestine under the influence of bile (Thompson & McManus, 2001). Because of this, we consider *Echinococcus* spp. eggs as not able to pass the digestive tract of a dog morphologically unaffected. A study suggested *Echinococcus* spp. eggs as likely capable of passing the digestive tract of a dog (Ziadinov et al., 2008). However, to our knowledge, the assumption made in that study has not been experimentally proven.

Humans are the definitive hosts of *Taenia solium* (Mendlovic et al., 2014). Following ingesting, *T. solium* oncospheres liberate from their envelopes (Mendlovic et al., 2014). This process is activated by bile salts (Mendlovic et al., 2014). After liberation the oncospheres invade the intestinal mucosa (Mendlovic et al., 2014). We believe that something similar may occur with other *Taenia* species eggs. Therefore, we consider *Taenia* spp. eggs as not capable of passing the digestive tract of a dog. If *Taenia* spp. eggs and *Echinococcus* spp. eggs are found by faecal examination, it is thus considered a patent infection.

Common *Cystoisospora* species in dogs are *I. canis* and *I. ohioensis* and in cats *I. felis* and *I. rivolta* (Taylor et al., 2007). These species are host-specific (Taylor et al., 2007). Rodents and ungulates can act as facultative paratenic hosts for several *Cystoisospora* species (Claerebout, 2008). Monozoic cysts of *Cystoisospora* spp. may be formed in the extraintestinal tissues of a paratenic host and these cysts may remain in the tissues for the life of the host (Claerebout, 2008; Dubey et al., 2009). Unsporulated oocysts of *Cystoisospora* spp. are excreted in the faeces of a dog or cat (Dubey et al., 2009). In the environment the oocysts need to sporulate forming sporozoites to become infective (Dubey et al., 2009). Sporulated oocysts are environmentally resistant and can survive for many months (Companion Animal Parasite Council, 2013a; Dubey et al., 2009). Following intake of oocysts by dogs, excystation of sporulated oocysts occurs in the intestinal lumen triggered by the presence of bile (Dubey et al., 2009). Therefore, we consider sporulated *Cystoisospora* spp. oocysts as not able to remain recognizable while passing the digestive tract of a dog. Unlike sporulated oocysts, we expect that unsporulated *Cystoisospora* spp. oocysts can pass the gastrointestinal tract of a dog and consequently this may result in a faecal sample false positive for patent infection. This expectation is partially based on our lab results; we frequently found unsporulated *Eimeria* spp. oocysts in the faeces of a dog examined in our laboratory. In conclusion, we assume that unsporulated *Cystoisospora* spp. oocysts found in a faecal sample (or sporulated *Cystoisospora* spp. oocysts, if sporulation already occurred by the time faecal examination is performed) can be the result of intestinal passage after coprophagia.

Giardia duodenalis (syn. *Giardia intestinalis*, *Giardia lamblia*) infects a wide range of mammalian hosts (Patton, 2013). The species can be divided into at least eight genetically distinct groups or assemblages (A to H) (Robertson, 2013; Ryan & Caccio, 2013).

Some assemblages have narrow host ranges, like assemblage C and D in dogs and assemblage F in cats, whereas other assemblages have broad host specificity, including humans and various animal species (assemblage A and B) (Patton, 2013). Dogs can carry different assemblages of *G. duodenalis* which are potentially pathogenic for humans (Patton, 2013; Robertson, 2013). However, dogs play a yet undefined role in the transmission of *G. duodenalis* to humans (Robertson et al., 2000).

Excreted cysts of *G. duodenalis* are immediately infective and are often intermittently shed (Patton, 2013; Ryan & Caccio, 2013). The cyst wall enables *G. duodenalis* cysts to survive for several weeks in the environment (Robertson, 2013; Ryan & Caccio, 2013). Following ingestion, excystation occurs in the duodenum resulting in the release of two trophozoites (Robertson, 2013; Ryan & Caccio, 2013). Exposure to factors like gastric acid, pepsin and the alkaline environment of the small intestine are thought to be the necessary stimuli for excystation of the cysts (Robertson, 2013; Ryan & Caccio, 2013). Because of the initiated excystation, *G. duodenalis* cysts are considered as not capable in passing the gastrointestinal tract of a dog without being morphologically affected. Therefore, a dog is considered patently infected if the dog's faecal sample tests positive for *G. duodenalis*.

1.4 The possible effects of coprophagia on faecal examination

As mentioned before, we consider *Toxocara* spp. eggs, *Trichuris* spp. eggs, strongyle-type eggs, *Capillaria* spp. eggs and unsporulated *Cystoisospora* spp. oocysts as able to remain recognizable while passing the digestive tract of a dog. These eggs and oocysts observed in a faecal sample may thus originate from intestinal passage after coprophagia. As a consequence, the results of faecal examination can be false positive for patent infections. Furthermore, several non-dog parasites may be difficult to distinguish from canine-specific parasites (Nijse et al., 2014; Sager et al., 2006).

Prevalence estimates of patent helminth infections in dogs, reported in cross-sectional studies, may be overestimated due to coprophagia (Fahrion et al., 2011; Nijse et al., 2014; Sager et al., 2006). Coprophagia can result in unnecessary deworming practices if deworming is based on a single faecal examination and assumptions on anthelmintic resistance may be related to coprophagia (Fahrion et al., 2011). In addition, control measures are often based on prevalence studies (Oliveira-Sequeira et al., 2002).

It is recommended to examine a second faecal sample to differentiate between passage after coprophagia and patent infection. To exclude a positive result due to coprophagia, the dog owner has to prevent the dog from eating faeces for three days. This period of three days is based on a study of Boillat et al. (2010), who investigated the gastrointestinal transit times in 31 healthy dogs (Boillat et al., 2010). Subsequently, the dog owner has to send the second faecal sample (also referred as confirmation sample). If this second faecal sample tests positive for the same type of helminth eggs or protozoan oocysts, then it can be considered a patent infection. If this second faecal sample tests negative, then the first sample can be considered as positive due to coprophagia.

1.5 Dogs as possible mechanical vectors

Coprophagic behavior can also pose a risk for people in the direct environment of dogs. For example, a *Toxocara* infection may be passed from animals to humans, causing human toxocarosis (Fahrion et al., 2011). Dogs with a patent *T. canis* infection can transmit *T. canis* to humans by shedding eggs with their faeces into the environment (Fahrion et al., 2011).

However, uninfected dogs may also pose a risk if they consume faeces from other dogs, foxes or cats. If uninfected dogs consume faeces containing *Toxocara* spp. eggs uncontaminated areas can become contaminated after intestinal passage. Coprophagic behavior in dogs can thus result in the dissemination of (zoonotic) intestinal parasites in the environment because dogs may serve as transport hosts.

1.6 The aim of this study

Research regarding the association between coprophagia and the prevalence of household dogs shedding helminth eggs and/or (oo)cysts is sparse. Nijssen et al. (2014) showed a significant association between reported coprophagia and the number of negative confirmation samples for *Toxocara* spp. eggs. However, that study focused only on *Toxocara* spp. eggs. Therefore, the aim of this study was to investigate to what extent coprophagia interferes with the prevalence of dogs shedding eggs of *Toxocara* spp., *T. vulpis*, strongyle type and *Capillaria* spp. in faecal samples of a group of adult household dogs in the Netherlands. For Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts the association between not-verified positive (or negative) samples and reported coprophagia was evaluated.

The following hypotheses have been formulated:

H0 = There is no significant association between reported coprophagia and the number of (confirmation) samples tested negative for helminth eggs and/or (oo)cysts.

H1 = There is a significant association between reported coprophagia and the number of (confirmation) samples tested negative for helminth eggs and/or (oo)cysts.

2. Materials and Methods

2.1 Design of the study

This research was part of a longitudinal study which takes place at the Faculty of Veterinary Medicine of Utrecht University with the purpose to gain more knowledge about patent *Toxocara canis* infections in dogs. Dog owners with a dog older than 6 months, living in the Netherlands could participate. Dog owners were asked to send a faecal sample monthly and to complete a questionnaire. The faecal samples were examined by the Centrifugal Sedimentation Flotation technique. Participants received the results of the faecal examination and were not treating their dogs with an anthelmintic unless it was considered necessary by the project team (eggs found by the lab, lactating bitches or traveling abroad).

2.2 Dog population

A total of 916 dogs participated in this study. Dog owners with a dog older than 6 months and living in the Netherlands could subscribe voluntarily.

2.3 Questionnaires

At the start of enrollment and on monthly base participants were requested to fill in an online questionnaire. Dog owners were asked about the dog (e.g. age, gender, breed, neutering status, health), the dog's habitat and management factors such as diet, walking environment, off-leash time outside, removal of faeces and anthelmintic treatment history. Especially important for this study was the question concerning the owner's perception of the coprophagic behavior of the dog.

2.4 The submitted faecal samples

Every month fresh faecal samples were collected by the dog owners and sent to the laboratory. The dog owners received a plastic bag to collect the faeces. To send the faeces safely to the laboratory, dog owners received a plastic box, a seal bag, a tissue and a bubble envelope. The majority of the samples were submitted by mail. However it was also possible to put the samples in a container at the laboratory. Every dog owner participating in the study had an unique number and the dog owners had to write this number and the name of the dog(s) on the plastic box(es) with the faecal sample(s).

2.5 Registration

Before the samples were analyzed, the incoming faecal samples were registered. The current date was written on the plastic boxes with the faecal samples and each box received a laboratory number (starting every week with number 1 on Monday).

2.6 Faecal examination

Usually the faecal samples were examined on the day of receiving them. If the samples were not immediately examined, they were stored in a refrigerator until examination within a maximum of 3 days. For logistic reasons, faecal samples of two dogs were initially pooled. In the case of a positive pooled sample, the Veterinary Microbiological Diagnostic Centre (VMDC) retested the two samples individually to identify which of the two dogs excretes helminth eggs and/or (oo)cysts. The VMDC used the Centrifugal Sedimentation Flotation technique with zinc sulfate solution (density: 1,34 g/cm³).

A sample was immediately examined individually in the case of a 'control sample', a 'second sample' or if the submitted sample was considered too small (sample with a weight less than 6 g). Two weeks after an anthelmintic treatment, a faecal sample was examined in order to check the efficacy of the anthelmintic. This sample is called a 'control sample'. A 'second sample' (also referred as 'confirmation sample') is taken to confirm a patent infection and to exclude a positive result due to coprophagia. This faecal sample is collected after the dog owner had to prevent the dog from eating faeces for a period of three days.

The faecal samples were first examined for consistency and macroscopically assessed for the detection of proglottids and adult helminths. Afterwards, the samples were examined by the Centrifugal Sedimentation Flotation technique. From each sample 3-5 g of faeces was suspended in water. In case of a single faecal sample, the sample was suspended in +/- 55 ml tap water and in case of a pooled sample, the sample was suspended in +/- 110 ml tap water. Subsequently, the suspension was strained through a tea strainer to remove large debris and placed in a centrifuge tube. The strained suspension was centrifuged for 2 minutes at 3000 rpm after which the supernatant was decanted. The flotation medium (sucrose solution with a specific gravity of 1,27-1,30 g/cm³) was added to the sediment until the tube was filled for approximately one fifth and the sediment was suspended thoroughly using a vortex. The tube was placed back in the centrifuge and filled completely to form a positive meniscus on top with additional flotation solution. A coverslip was placed on top and the tube was centrifuged for 2 minutes at 3000 rpm. The cover slips were removed from the centrifuge tubes and placed on microscope slides.

2.7 Microscopic analysis

The slides were examined by light microscopy. The entire slide was examined systematically for helminth eggs and (oo)cysts by using the 40x and 100x magnification. A few rows of visual fields were observed at 400x magnification to detect (oo)cysts like *Giardia* cysts. All eggs, oocysts, cysts and larvae found in the faecal samples (including typical non-dog parasites) were identified according to morphological characteristics and if considered necessary, microscopically measured using an ocular with an integrated micrometer scale. *Toxascaris leonina*, *Sarcocystis* spp., *Hammondia heydorni* and *Neospora caninum* were not included in this study because of their low prevalence estimates. However, if eggs or oocysts of these parasites were found in a faecal sample, they were identified and reported. Differentiation between *H. heydorni* and *N. caninum* oocysts was not possible by microscopy due to morphological similarity. Because of this, they were reported as *Neospora/Hammondia* oocysts. *Cystoisospora* spp. oocysts found in a faecal sample were difficult to distinguish from *Eimeria* spp. oocysts. If an oocyst had a micropyle or if the oocyst was sporulated and contained four sporocysts with each two sporozoites, it certainly was an *Eimeria* spp. oocyst (Companion Animal Parasite Council, 2013a; Constable, 2012). Sporulated *Cystoisospora* spp. oocysts are characterized by having two sporocysts, each containing four sporozoites (Dubey et al., 2009). An unsporulated oocyst without a micropyle might be a *Cystoisospora* spp. oocyst, however this could not be said with certainty. Furthermore, the eggs of *Taenia* spp. could not be differentiated from those of *Echinococcus* spp. based on morphological characteristics (Conboy, 2009a). In this study no other diagnostic techniques were used to identify *Taenia* or *Echinococcus* species. That is why we speak of the family of Taeniidae.

Also *Toxocara* spp. eggs found in a faecal sample could not be distinguished by microscopy. If *Toxocara* spp. eggs were found, the major axes of the eggs were measured. However, as mentioned in the introduction egg measurement is not a good criterion to differentiate between *T. canis* and *T. cati* (Uga et al., 2000). That is why a faecal sample with *Toxocara* spp. eggs was considered positive regardless of the egg size.

If *Trichuris* spp. eggs, *Capillaria* spp. eggs or strongyle-type eggs were found in a sample, the samples were considered positive based on egg measurement. The samples were positive if *Trichuris* spp. eggs had the size of *T. vulpis* (70-90 x 30-40 µm), if *Capillaria* spp. eggs had the size of *C. aerophila* (59-80 x 30-40 µm) or *C. boehmi* (54-60 x 30-35 µm), or if strongyle-type eggs fit the size of the canine hookworms *U. stenocephala* (71-93 x 35-58 µm) or *A. caninum* (56-75 x 34-47 µm) (Claerebout, 2008; Companion Animal Parasite Council, 2012b; Conboy, 2009b; Taylor et al., 2007). However, if a low number of strongyle type of eggs (size: +/- 80 µm) were found in combination with many *Eimeria* spp. oocysts, then the pooled sample was not retested individually, because the sample was very suspicious of being positive due to coprophagia. The same applies for a sample with a few *Capillaria* spp. eggs in combination with *Heterakis* spp. eggs. Ideally, each pooled sample positive for dog-typical parasites should be tested individually. Due to practical constraints this was not possible in this study. Table 2 shows the score system used to give an estimation of the number of eggs and/or (oo)cysts found in a microscope slide.

Table 2 Score system used for the quantity of eggs and/or (oo)cysts

Score	Description
-	No eggs or (oo)cysts
+	A few eggs or (oo)cysts in the total slide
++	One egg or (oo)cyst in almost every field (at 40x magnification)
+++	Full of eggs or (oo)cysts

2.8 Positive results due to coprophagia

The result of the faecal examination may be false positive for patent infection due to intestinal passage after coprophagia. *Toxocara* spp. eggs, *Trichuris* spp. eggs, strongyle-type eggs, *Capillaria* spp. eggs and unsporulated *Cystoisospora* spp. oocysts were considered as able to remain recognizable while passing the digestive tract of a dog. If these helminth eggs were found in a faecal sample of a dog, we asked the owner to prevent the dog from eating anything from the ground for three days and subsequently to submit a second faecal sample. If this second faecal sample tested positive for the same type of helminth eggs, then the dog was considered patently infected. If this second faecal sample tested negative, then the first sample was considered false positive for patent infection due to coprophagy. Unfortunately, in this study, no confirmation sample was asked if (un)sporulated *Cystoisospora* spp. oocysts were found in a faecal sample, mainly because of limited clinical relevance in adult dogs.

A dog can also be considered as being coprophagic if the faecal examination showed typical non-dog parasite eggs and/or oocysts, like: *Eimeria* spp. oocysts, strongyle-type eggs that did not fit the egg size of canine hookworms, *Moniezia* spp. eggs, *Anoplocephala* spp. eggs and *Heterakis/Ascaridia* spp. eggs (Nijssen et al., 2014).

2.9 Statistical analyses

The Faculty of Veterinary Medicine maintains a database in which the results of the questionnaires and the faecal examinations are collected. This study used the results of the faecal samples examined during my research period in the laboratory (February 3, 2014 until April 4, 2014). Because of the limited period of time, data obtained during the period of March 1, 2012 to March 1, 2013 were also used. Regarding reported coprophagia, this study used data obtained from the questionnaires completed by the dog owners at the start of the longitudinal study.

The percentages of samples positive for *Toxocara* spp., *T. vulpis*, hookworms, *Capillaria* spp., Taeniids, *Cystoisospora* spp. and *G. duodenalis* at the first examination were determined as the number of positive faecal samples among the total number of examined faecal samples. The percentages of dogs whose faeces tested positive over a longer period of time at least once for *Toxocara* spp. eggs, *T. vulpis* eggs, strongyle-type eggs, *Capillaria* spp. eggs, Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts were also calculated. If *Toxocara* spp. eggs, *T. vulpis* eggs, strongyle-type eggs and/or *Capillaria* spp. eggs were found at the first examination, a second faecal sample was asked. For each parasite, the proportion of negative confirmation samples was calculated and a Pearson Chi-Square was used to evaluate the association between reported coprophagia and the result (positive or negative) of the confirmation sample. If the expected frequency in any one of the four cells of a contingency table was less than five, a Fisher's Exact Test was applied (Petrie & Watson, 2006). In addition, a binomial probability test was used to evaluate in the group of negative second samples the difference between the proportion of dogs with reported coprophagic behavior and the proportion of dogs that do not eat faeces according to the owner. A P-value < 0.05 was considered statistically significant. For Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts, a Pearson Chi-Square or Fisher's Exact Test evaluated the association between not-verified positive (or negative) samples and reported coprophagia. From some dogs, data were missing and consequently the results from these dogs were excluded from the data analyses. The statistical analyses were performed using SPSS 22.0 software for Windows.

3. Results

3.1 Questionnaire results

Since the longitudinal study started in 2012, we received at least one sample from 916 participating dogs. Questionnaire results showed that 399 of the 916 (43,6%) dogs are eating faeces according to their owner (Figure 1). The owners of 513 of the 916 (56,0%) dogs mentioned that their dog is not coprophagic and four dog owners did not know whether their dog eats faeces. Of the dogs that do not eat faeces according to their owner, 250 (48,7%) walk off the leash for more than 50 percent of the time. In addition, 33 of the 58 (56,9%) faecal samples positive for *Eimeria* spp. oocysts, originated from dogs that do not consume faeces according to their owner.

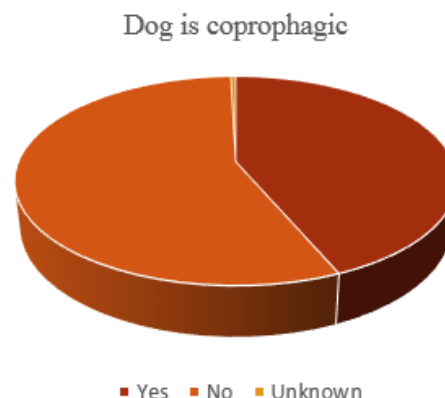


Fig. 1 Prevalence of coprophagia

3.2 Results obtained during the period of March 1, 2012 to March 1, 2013

The results obtained during the period of March 1, 2012 to March 1, 2013 will be presented first.

3.2.1 Prevalence of helminth eggs and (oo)cysts

Between March 1, 2012 and March 1, 2013, a total of 4840 faecal samples originating from 892 Dutch household dogs were examined for the presence of intestinal parasites. The number of samples and the percentages of samples positive for *Toxocara* spp., *T. vulpis*, hookworms, *Capillaria* spp., Taeniids, *Cystoisospora* spp. and *G. duodenalis* are presented in Table 3. *Toxocara* spp. eggs, strongyle-type eggs and *G. duodenalis* cysts were most frequently found. Low percentages were found for the eggs of *T. vulpis*, *Capillaria* spp. and Taeniids.

Table 3 Helminth eggs and (oo)cysts found in the faecal samples and their percentage

Species	Number of positive samples*	Percentage (%)
<i>Toxocara</i> spp.	272	5,62
<i>Trichuris vulpis</i>	34	0,70
Hookworms	120	2,48
<i>Capillaria</i> spp.	26	0,54
Taeniids	22	0,45
<i>Cystoisospora</i> spp.	82	1,70
<i>Giardia duodenalis</i>	110	2,27

* These are the results of the first examination

Between March 1, 2012 and March 1, 2013, the percentages of dogs whose faeces tested at least one time positive for *Toxocara* spp. eggs, *T. vulpis* eggs, strongyle-type eggs, *Capillaria* spp. eggs, Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts, were (48/892=) 5.4%, (9/892=) 1.0%, (21/892=) 2.4%, (10/892=) 1.1%, (3/892=) 0.3%, (11/892=) 1.2% and (12/892=) 1.3% respectively.

3.2.2 False positive samples due to intestinal passage after coprophagia

If a sample tested positive for *Toxocara* spp. eggs, *T. vulpis* eggs, strongyle-type eggs and/or *Capillaria* spp. eggs at the first examination, the owner was asked to send a second faecal sample after keeping the dog from eating faeces for three days. In this way we were able to differentiate between passage after coprophagia and a patent infection. The association between reported coprophagy and the result of the confirmation sample was evaluated. In 272 of the 4840 (5,6%) faecal samples *Toxocara* spp. eggs were found. Unfortunately we did not receive a second sample in all 272 cases. We received 137 confirmation samples (Table 4, Figure 2). Among these, 56 (40,9%) tested negative and consequently the related first samples were considered false positive for patent infection.

Table 4 Results of the second faecal samples examined for *Toxocara* spp. eggs

<i>Toxocara</i> spp.	Dog eating faeces	Dog does not eat faeces	Blank*	Total
Positive second sample	46	34	1	81
Negative second sample	34	22	0	56
Total	80	56	1	N = 137

* Dog owners did not answer the question about coprophagy

A Pearson Chi-Square showed no significant association between reported coprophagic behavior and the result of the confirmation sample ($\chi^2 = 0,141$, $df = 1$, $p = 0,708$). There seemed to be a difference between the proportions of coprophagic dogs and not coprophagic dogs in the group of negative second samples ($34/56 = 60,7\%$ versus $22/56 = 39,3\%$), however this difference is not significant according to the binomial probability test ($p = 0,141$).

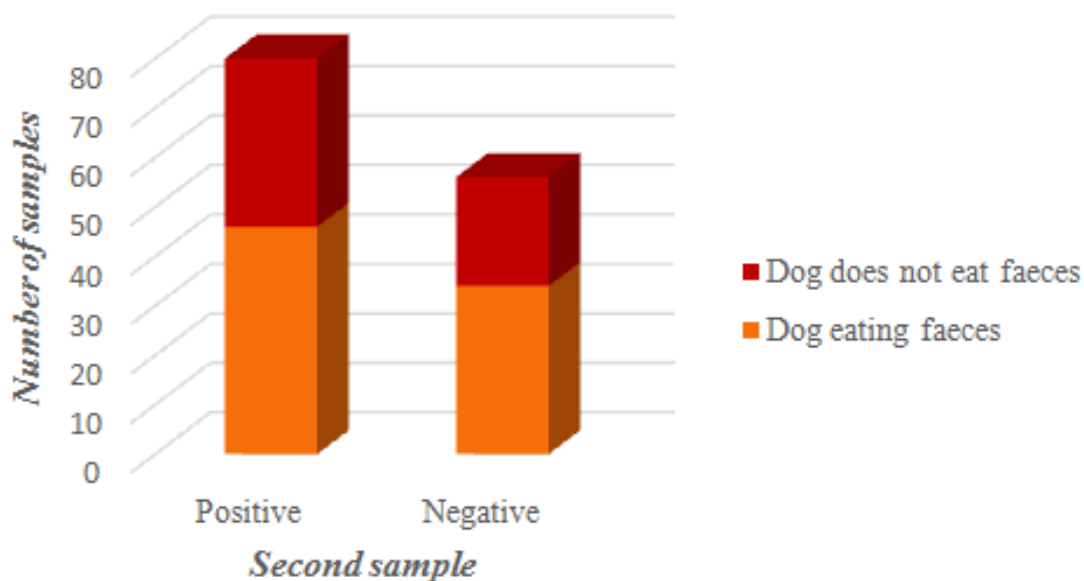


Fig. 2 Results of the second faecal samples examined for *Toxocara* spp. eggs

From the 4838 faecal samples, 120 (2,5%) tested positive for strongyle type of eggs with the size of *U. stenocephala* and/or *A. caninum*. Unfortunately, only 23 confirmation samples were collected and examined. Of these, 16 (69,6%) tested negative (Table 5). Two cells of the contingency table had an expected count less than five. Because of this, a Fisher's Exact Test was applied. No significant association was found between the result of the confirmation sample and reported coprophagia ($p = 0,345$). In the group of negative confirmation samples, the percentage of dogs being coprophagic according to the owner was not significantly different ($p = 0,804$) from the percentage of dogs that do not eat faeces ($9/16 = 56,3\%$ versus $7/16 = 43,7\%$).

Table 5 Results of the second faecal samples examined for strongyle-type eggs

Hookworms	Dog eating faeces	Dog does not eat faeces	Total
Positive second sample	6	1	7
Negative second sample	9	7	16
Total	15	8	N = 23

Of the 4838 faecal samples, 34 (0,7%) were found positive for *T. vulpis*. Only 13 confirmation samples were collected and analyzed. Among these, 7 (53,8%) were negative and consequently it was assumed that these dogs did not have a patent *T. vulpis* infection (Table 6). A Fisher's Exact Test showed no significant association between the outcome of the second sample and reported coprophagic behavior ($p = 0,592$). Moreover, in the group of negative second samples a binomial probability test showed no significant difference between the proportion of dogs with reported coprophagic behavior and the proportion of dogs that do not eat faeces according to the owner ($p = 1,000$). In 26 of the 4838 (0,5%) faecal samples *Capillaria* spp. eggs were detected. The number of second samples ($n=1$) was too low to perform valid statistical analyses.

Table 6 Results of the second faecal samples examined for *Trichuris vulpis* eggs

<i>Trichuris vulpis</i>	Dog eating faeces	Dog does not eat faeces	Total
Positive second sample	2	4	6
Negative second sample	4	3	7
Total	6	7	N = 13

For Taeniids eggs and *G. duodenalis* cysts it was not necessary to examine a second faecal sample, because we considered Taeniids eggs and *G. duodenalis* cysts as not capable of passing the gastrointestinal tract of a dog without being morphologically affected. It is recommended to examine a second sample if (un)sporulated *Cystoisospora* spp. oocysts are found in a faecal sample, but in this study dog owners were not asked to send a second sample. For Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts the association was evaluated between reported coprophagia and the result of the faecal sample at the first examination. There was no significant association between reported coprophagy and

the number of faecal samples tested positive (or negative) for Taeniids eggs ($p = 0,841$), *Cystoisospora* spp. oocysts ($p = 0,113$) and *G. duodenalis* cysts ($p = 0,083$). The results of the faecal samples examined for Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts can be seen in Appendix A. The statistical analyses performed using SPSS 22.0 software for Windows can be found in Appendix B.

3.3 Results obtained during the period of February 3, 2014 until April 4, 2014

Next, the results obtained during my research period are presented.

3.3.1 Prevalence of helminth eggs and (oo)cysts

During my research period in the lab, a total of 871 faecal samples originating from 518 Dutch household dogs were examined for the presence of helminth eggs and/or (oo)cysts. The helminth eggs and protozoan (oo)cysts found in the faecal samples at the first examination are presented in Table 7. In agreement with our results reported in section 3.2.1, *Toxocara* spp. eggs, strongyle-type eggs and *G. duodenalis* cysts were most commonly found.

Table 7 Eggs and (oo)cysts found in the faecal samples at the first examination

Species	Number of positive samples	Percentage (%)
<i>Toxocara</i> spp.	48	5,51
<i>Trichuris vulpis</i>	4	0,46
Hookworms	15	1,79
<i>Capillaria</i> spp.	6	0,71
Taeniids	4	0,46
<i>Cystoisospora</i> spp.	7	0,81
<i>Giardia duodenalis</i>	28	3,21

Between February 3, 2014 and April 4, 2014, the percentages of dogs whose faeces tested at least one time positive for *Toxocara* spp. eggs, *T. vulpis* eggs, strongyle-type eggs, *Capillaria* spp. eggs, Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts, were (29/518=) 5.6%, (3/518=) 0.58%, (8/505=) 1.6%, (5/505=) 1.0%, (2/518=) 0.4%, (7/510=) 1.4% and (18/518=) 3.5% respectively.

3.3.2 False positive samples due to intestinal passage after coprophagia

A second faecal sample was asked if *Toxocara* spp. eggs, *T. vulpis* eggs, hookworm eggs and/or *Capillaria* spp. eggs were found at the first examination. Unfortunately, the number of confirmation samples for both *T. vulpis* ($n=2$) and *Capillaria* spp. ($n=1$) was too low to perform valid calculations.

Of the 871 faecal samples, 48 (5,5%) tested positive for *Toxocara* spp. eggs at the first examination. A total of 35 confirmation samples were collected and examined (Table 8). Of these, 14 (40%) tested negative at the second examination and consequently the related first samples were considered false positive for patent infection. A Pearson Chi-Square showed a significant association between a dog being (or being not) coprophagic according to the owner and the result of the confirmation sample ($\chi^2 = 3.998$, $df = 1$, $p = 0.046$). In the group of negative confirmation samples, the percentage of coprophagic dogs was slightly smaller than the percentage of dogs that do not eat faeces according to the owner (6/14 = 42,9% versus 8/14 = 57,1%). The binomial probability test found no significant difference ($p = 0,791$) between these proportions.

Table 8 Results of the second faecal samples examined for *Toxocara* spp. eggs

<i>Toxocara</i> spp.	Dog eating faeces	Dog does not eat faeces	Total
Positive second sample	16	5	21
Negative second sample	6	8	14
Total	22	13	N = 35

Of the 871 samples, 32 were part of a pooled sample that tested positive for strongyle type of eggs with the size of *U. stenocephala* and/or *A. caninum*, but were not retested individually. Consequently, these samples were excluded from the calculations. In 15 of the 839 (1,8%) faecal samples strongyle type of eggs were found with the size of canine hookworms. Unfortunately, we received only 8 confirmation samples (Table 9). In all the confirmation samples (100%) no strongyle-type eggs were found. Because of the low number of second samples, valid statistical analyses were not performed.

Table 9 Results of the second faecal samples examined for strongyle-type eggs

Hookworms	Dog eating faeces	Dog does not eat faeces	Total
Positive second sample	0	0	0
Negative second sample	4	4	8
Total	4	4	N = 8

As mentioned before, no confirmation sample was asked if a sample tested positive for Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts. A Pearson Chi-Square or a Fisher's Exact Test was used to evaluate the association between reported coprophagy and the (not-verified) result of the faecal sample. No significant association was present between a dog being (or being not) coprophagic and the number of samples positive (or negative) for Taeniids eggs ($p = 0,326$), *Cystoisospora* spp. oocysts ($p = 1,000$) and *G. duodenalis* cysts ($p = 0,225$). The results of the faecal samples examined for Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts during the period of February 3, 2014 until April 4, 2014 can be found in Appendix C. The performed statistical tests can be shown in Appendix D.

4. Discussion and conclusions

4.1 Prevalence of coprophagia

In the present study the percentage of dogs eating faeces according to their owner was 43,6%. This is in agreement with an earlier reported prevalence of coprophagia (44,7%) in a study of van der Borg et al. (2006). The prevalence of coprophagia reported in this study is based on the questionnaires completed by the dog owners. However, whether this prevalence of reported coprophagia corresponds with the true prevalence of dogs being coprophagic is questionable. The results of the faecal examination showed that 56,9% of the faecal samples positive for *Eimeria* spp. oocysts originated from dogs that do not consume faeces according to their owner. *Eimeria* spp. do not infect dogs, so these dogs have eaten faeces from other animal species. In addition, 48,7% of the non-coprophagic dogs walk off the leash more than 50 percent of the time. In these cases we can imagine that the perception of the dog owners might be less reliable. Finally, our results showed that a considerable percentage of the negative confirmation samples, which were considered false positive due to intestinal passage after coprophagia, originated from dogs that do not eat faeces according to their owner.

4.2 Prevalence of dogs shedding helminth eggs and/or (oo)cysts

For the period of March 1, 2012 to March 1, 2013, the percentages of samples positive for *Toxocara* spp., *T. vulpis*, hookworms (*U. stenocephala* and *A. caninum*), *Capillaria* spp., Taeniids, *Cystoisospora* spp. and *G. duodenalis* were calculated. In addition, the prevalence estimates of dogs whose faeces tested positive over a longer period at least once for the different helminth eggs and/or protozoan (oo)cysts were determined. These calculations were also performed for the faecal samples examined during my research period in 2014. *Toxocara* spp. eggs, strongyle-type eggs and *G. duodenalis* cysts were most commonly found. We did not compare the prevalence estimates found in this study with those of other reports, because prevalence estimates are usually based on cross-sectional studies and this was a longitudinal study.

It is important to realize that the Centrifugal Sedimentation Flotation (CSF) technique as sole screening technique has its limitations. The CSF technique is not very sensitive for all the intestinal parasites examined in this study and other diagnostic techniques (like PCR and ELISA) were not included in this study. For example, the percentage of faecal samples positive for *G. duodenalis* cysts may be underreported because of their intermittent shedding, the limited sensitivity of the CSF technique to detect *Giardia* spp. cysts, the examination of a few rows of the microscope slide at 400x magnification and the analysis of a single faecal sample (three faecal samples collected throughout 3-5 days may be recommended) (Claerebout et al., 2009; Patton, 2013). Furthermore, this study almost certainly underreported the percentage of samples positive for Taeniids eggs because Taeniids eggs are focally distributed in the faeces of a dog, shed in an irregular pattern and the CSF technique has a poor detection sensitivity for Taeniids eggs (Companion Animal Parasite Council, 2013b; Conboy, 2009a). If a pooled sample tested positive for Taeniids eggs, Taeniids eggs were not always detected if the samples were retested individually. The CSF technique can be considered reliable for the finding of *Cystoisospora* spp. oocysts, hookworm eggs, *Capillaria* spp. eggs, *T. vulpis* eggs and *Toxocara* spp. eggs (Companion Animal Parasite Council, 2013a; Conboy, 2009b; Overgaauw, 1997).

Besides the technique used in this study, other factors may have influenced the reported percentages of samples positive for helminth eggs and/or (oo)cysts. For example, the prevalence estimates are likely to be underestimated due to the exclusion of dogs younger than 6 months of age. Most helminth and protozoa infections are more prevalent in young dogs (Little et al., 2009). Although, *T. vulpis* infections are more common in older dogs (Little et al., 2009). Furthermore, the percentage of faecal samples positive for *Cystoisospora* spp. oocysts may be underreported or over-reported, because unsporulated *Cystoisospora* spp. oocysts can be difficult to differentiate from *Eimeria* spp. oocysts. Finally, we almost certainly underreported the percentages of samples positive for *Capillaria* spp. eggs and hookworm eggs because not all pooled samples positive for *Capillaria* spp. eggs and strongyle-type eggs were examined individually due to practical constraints. If a low number of *Capillaria* spp. eggs were found in combination with *Heterakis* spp. eggs, the *Capillaria* spp. eggs in the dog's faeces were likely to be the result of eating bird faeces. Consequently, these pooled samples positive for *Capillaria* spp. eggs were not retested individually. The same applies for a sample with a few strongyle-type eggs (with the size of canine hookworms) and many *Eimeria* spp. oocysts. These strongyle-type eggs may be the result of a dog eating rabbit faeces, because the strongyle-type eggs in rabbit faeces fit the size of canine hookworms eggs (Nijse et al., 2014).

Generally, it is important to keep in mind that the finding of helminth eggs and/or (oo)cysts in the faeces of a dog does not necessarily mean that the dog is infected. A false positive diagnosis for infection with intestinal parasites may be the result of passive passage, the finding of morphological similar non-dog parasites or environmental contamination. Definitive diagnosis of patent helminth infections in dogs requires the finding of adult worms (e.g. in the dog's faeces after an anthelmintic treatment).

4.3 Capability of passing the gastrointestinal tract of a dog

Eggs and (oo)cysts may be excreted in the dog's faeces while the dog is not infected, due to intestinal passage after coprophagia. To achieve a false positive diagnosis for patent infection, the stages must be recognizable as egg or (oo)cyst after passing the gastrointestinal tract of a dog. In this study, we assumed that *Toxocara* spp. eggs, *Trichuris* spp. eggs, strongyle-type eggs, *Capillaria* spp. eggs and unsporulated *Cystoisospora* oocysts are able to pass the digestive tract of a dog. *Taenia* spp. eggs, *Echinococcus* spp. eggs, sporulated *Cystoisospora* oocysts and *G. duodenalis* cysts were considered as not able to pass the gastrointestinal tract of a dog. Research is needed in which dogs without helminth infection ingest non-infective stages of helminth eggs and (oo)cysts, to get more evidence whether these eggs and (oo)cysts can pass the gastrointestinal tract of a dog morphologically unaffected. It may be possible that for example *G. duodenalis* cysts are able to pass the gastrointestinal tract of a dog if they are not exposed to the necessary stimuli for excystation (e.g. if cysts are hidden in a bulk faeces).

4.4 The possible effects of coprophagia on the results of faecal examination

Research regarding the association between coprophagia and the prevalence of helminth eggs and/or (oo)cysts found in dog's faeces is sparse. Recently, the study of Nijse et al. (2014) concluded that prevalence estimates for patent helminth infections in dogs reported in cross-sectional prevalence studies need to be interpreted with caution, because they are usually based on the examination of a single faecal sample. Nijse et al. (2014) showed a significant

association between reported coprophagia and the number of negative confirmation samples for *Toxocara* spp. eggs.

4.4.1 The percentage of negative confirmation samples

In this study, the percentage of negative confirmation samples was 40,9% for *Toxocara* spp. eggs, 69,6% for strongyle-type eggs and 53,8% for *T. vulpis* eggs during the period of March 1, 2012 to March 1, 2013. These results match reasonably with those observed in the study of Nijssen et al. (2014). During my research period in 2014, 40% of the confirmation samples for *Toxocara* spp. eggs were negative and the percentage of negative confirmation samples for strongyle-type eggs was 100%.

A confirmation sample of a patently infected dog can be negative due to intermittent shedding of eggs. *U. stenocephala* eggs, *Capillaria* spp. eggs and *T. vulpis* eggs can be shed intermittently (Conboy, 2009b; Nijssen et al., 2014; Traversa, 2011). According to Nijssen et al. (2014), intermittent shedding has not been described for infections with *T. canis* in adult dogs. Furthermore, if a low number of eggs is present in the faeces due to a starting low patent infection or a final stage of patent infection, it could be possible that no eggs are found in the examined 3-5 gram faeces. However, in such a case the dog should have tested positive in the following or preceding month(s) (Nijssen et al., 2014). Other possible explanations are a low egg production or an inhomogeneous distribution of worm eggs in a faecal sample. However, these explanations are not likely to cause such high numbers of negative confirmation samples. The results indicate that a considerable percentage of samples positive for *T. canis*, *T. vulpis* and hookworms at the first examination were false positive for patent infection. Nijssen et al. (2014) suggested that coprophagia could be the cause of the high number of negative confirmation samples. In this study, we differentiated between intestinal passage after coprophagia and patent infection by the examination of a second faecal sample. The second sample (i.e. confirmation sample) was collected after a period of three days in which the dog owner prevented the dog from eating faeces.

4.4.2 The association with reported coprophagic behaviour

Nijssen et al. (2014) found significantly more negative confirmation samples for *Toxocara* spp. eggs in dogs eating faeces. Contrary to our expectations, in this study no significant association was observed between reported coprophagia and the number of negative confirmation samples for *Toxocara* spp. eggs, *T. vulpis* eggs and strongyle-type eggs. The results obtained during my research period in 2014 showed a significant association between reported coprophagia and the result (positive or negative) of the confirmation sample for *Toxocara* spp. eggs. However, in the group of negative confirmation samples, no significant difference was found between the percentage of coprophagic dogs and the percentage of dogs that do not eat faeces according to the owner.

The results of this study differ from the findings of Nijssen et al. (2014). These differences may be explained by the limited number of confirmation samples examined in this study. We examined just a small number of second samples during my research period in the lab: 8 for strongyle-type eggs and 35 for *Toxocara* spp. eggs. Because of the limited data, we decided to use also previously obtained data. However, during the period of March 1, 2012 to March 1, 2013, we also analyzed a relatively small number of second samples. For *T. vulpis* eggs we examined 13 confirmation samples and for strongyle-type eggs 23 samples. The number of second samples for *Capillaria* spp. was too low (n=1) to perform valid calculations.

If *Toxocara* spp. eggs were found in a faecal sample at the first microscopic examination, we received a confirmation sample in approximately 50% of the cases. The low percentage of examined second samples should be taken into account when interpreting these results.

As mentioned before, the owners' perception of the coprophagic behavior of their dog does not always correspond with the results of the faecal examination. It is possible that a confirmation sample was negative due to coprophagia while the dog owner reported the dog as not being coprophagic. Also a dog with coprophagic behaviour according to the owner does not have to eat faeces before every faecal examination. For example, in winter time it is possible that a dog eats faeces less frequently. Moreover, we found in some confirmation samples typical non-dog parasite eggs and/or oocysts, concluding that a dog owner does not always succeed in keeping the dog from eating faeces. As a consequence, some positive second samples might be false positive for patent infection. However, non-dog parasites may also be found in a positive confirmation sample while the dog has a concurrent patent infection. In conclusion, these results warrant cautious interpretation. More studies with a higher compliance of owners providing confirmation samples after keeping the dog from eating anything from the ground are needed for a reliable determination of the exact impact of coprophagia on the prevalence of dogs shedding helminth eggs and (oo)cysts.

For Taeniids eggs, *Cystoisospora* spp. oocysts and *Giardia duodenalis* cysts no significant association was found between the not-verified result of the faecal sample and reported coprophagia. Unsporulated *Cystoisospora* oocysts were considered able to pass the digestive tract of a dog. Further research is recommended, in which faecal samples positive for *Cystoisospora* oocysts also will be confirmed by examining a second sample.

4.5 Practical relevance

Intestinal parasites, both helminths and protozoa, may cause disease in dogs. Moreover, several canine intestinal parasites are zoonotic, which means they can be transmitted from animals to humans (Little et al., 2009). Zoonotic parasites such as *T. canis*, *Echinococcus* spp. and *G. duodenalis* are considered as a public health risk (Claerebout et al., 2009). To protect the health of both household dogs and humans, active monitoring of the prevalence of patent infections in dogs should be an ongoing task (Little et al., 2009; Oliveira-Sequeira et al., 2002). However, the high number of negative confirmation samples in this study indicate that the prevalence estimates for patent *T. canis*, *T. vulpis* and hookworm infections reported in cross-sectional studies may be overestimated. This is important to bear in mind if assumptions or control measures are based on prevalence studies. Repeated faecal examinations should be applied to confirm patent infections (Fahrion et al., 2011).

Control measures include coproscopic monitoring, anthelmintic treatments and hygienic measures to prevent or decrease contamination of the environment with eggs and (oo)cysts (Claerebout et al., 2009). Routine faecal examinations resulting in, if necessary, prompt treatments are recommended to control patent infections (Claerebout et al., 2009; Little et al., 2009). This study supports this recommendation. Our results showed that in the majority of the faecal samples no helminth eggs and/or oocysts were found. Consequently, if anthelmintic treatments are blindly administered, the majority of dogs will be treated unnecessary. Additionally, if practically possible, this study advises routine faecal examinations after keeping a dog from eating faeces for three days. In this way a false positive diagnosis for patent infection due to coprophagy can be excluded.

4.6 Conclusions

In conclusion, a remarkable number of samples positive for *T. canis* eggs, *T. vulpis* eggs and hookworm eggs returned as negative confirmation sample. The results indicate that a considerable percentage of infections with *T. canis*, *T. vulpis* and hookworms diagnosed by a single faecal examination may be false positive for patent infection. Consequently, prevalence rates estimated in cross-sectional studies may be overestimated and have to be interpreted with caution. Repeated coproscopic examinations to confirm patent infections are recommended, e.g. in epidemiological studies.

It is questionable whether the considerable percentage of negative confirmation samples is caused by intestinal passage after coprophagia. The reported prevalence of coprophagia (43,6%) in this study showed that coprophagia is not uncommon among dogs. The true prevalence of coprophagic dogs may even be higher. This study did not find a significant association between reported coprophagy and the number of negative confirmation samples for *Toxocara* spp., *Trichuris* spp. and hookworms. Furthermore, no significant association was present between a dog being (or being not) coprophagic and the number of samples positive (or negative) for Taeniids eggs, *Cystoisospora* spp. oocysts and *G. duodenalis* cysts. Consequently, the null hypothesis of no association between reported coprophagia and the number of negative (confirmation) samples is retained. More studies with a higher compliance of owners providing confirmation samples are needed for a reliable determination of the exact influence of coprophagia on the prevalence of household dogs shedding helminth eggs and (oo)cysts.

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Appendices

Appendix A: The results of the faecal samples examined for Taeniids eggs, *Cystoisospora* spp. oocysts and *Giardia* cysts during the period of March 1, 2012 to March 1, 2013

- Taeniids

Taeniids	Dog eating faeces	Dog does not eat faeces	Unknown	Blank	Total
Positive sample	9	12	0	1	22
Negative sample	2109	2574	20	112	4815
Total	2118	2586	20	113	N = 4837

- *Cystoisospora* spp.

<i>Cystoisospora</i> spp.	Dog eating faeces	Dog does not eat faeces	Unknown	Blank	Total
Positive sample	43	37	0	2	82
Negative sample	2073	2549	20	111	4753
Total	2116	2586	20	113	N = 4835

- *Giardia duodenalis*

<i>Giardia duodenalis</i>	Dog eating faeces	Dog does not eat faeces	Unknown	Blank	Total
Positive sample	57	50	1	2	110
Negative sample	2061	2536	19	111	4727
Total	2118	2586	20	113	N = 4837

Appendix B: The statistical analyses performed for the faecal samples examined during the period of March 1, 2012 to March 1, 2013

The statistical analyses were performed using SPSS 22.0 software for Windows:

- *Toxocara* spp. – Pearson Chi-Square & Binomial probability test

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,141 ^a	1	,708		
Continuity Correction ^b	,039	1	,843		
Likelihood Ratio	,141	1	,708		
Fisher's Exact Test				,727	,422
N of Valid Cases	136				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 23,06.

b. Computed only for a 2x2 table

Binomial Test

	Category	N	Observed Prop.	Test Prop.	Exact Sig. (2-tailed)	Exact Sig. (2-tailed)
coprophagy	Group 1	2,00	34	,61	,50	,141
	Group 2	1,00	22	,39		,141
	Total		56	1,00		

- Hookworms – Fisher's Exact Test & Binomial probability test

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,864 ^a	1	,172		
Continuity Correction ^b	,791	1	,374		
Likelihood Ratio	2,048	1	,152		
Fisher's Exact Test				,345	,190
N of Valid Cases	23				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 2,43.

b. Computed only for a 2x2 table

Binomial Test

	Category	N	Observed Prop.	Test Prop.	Exact Sig. (2-tailed)	Point Probability
coprophagy	Group 1	2,00	9	,56	,50	,804
	Group 2	1,00	7	,44		,175
	Total		16	1,00		

- *Trichuris vulpis* – Fisher’s Exact Test & Binomial probability test

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,737 ^a	1	,391		
Continuity Correction ^b	,090	1	,764		
Likelihood Ratio	,746	1	,388		
Fisher's Exact Test				,592	,383
N of Valid Cases	13				

a. 4 cells (100,0%) have expected count less than 5. The minimum expected count is 2,77.

b. Computed only for a 2x2 table

Binomial Test

	Category	N	Observed Prop.	Test Prop.	Exact Sig. (2-tailed)	Point Probability
coprophagy	Group 1	2,00	4	,57	,50	1,000
	Group 2	1,00	3	,43		,273
	Total		7	1,00		

- Taeniids – Pearson Chi-Square

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,040 ^a	1	,841		
Continuity Correction ^b	,000	1	1,000		
Likelihood Ratio	,040	1	,841		
Fisher's Exact Test				1,000	,511
N of Valid Cases	4704				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 9,46.

b. Computed only for a 2x2 table

- *Cystoisospora* spp. – Pearson Chi-Square

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2,516 ^a	1	,113		
Continuity Correction ^b	2,170	1	,141		
Likelihood Ratio	2,500	1	,114		
Fisher's Exact Test				,114	,071
N of Valid Cases	4702				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 36,00.

b. Computed only for a 2x2 table

- *Giardia duodenalis* – Pearson Chi-Square

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2- sided)	Exact Sig. (1- sided)
Pearson Chi-Square	3,007 ^a	1	,083		
Continuity Correction ^b	2,676	1	,102		
Likelihood Ratio	2,989	1	,084		
Fisher's Exact Test				,095	,051
N of Valid Cases	4704				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 48,18.

b. Computed only for a 2x2 table

Appendix C: The results of the faecal samples examined for Taeniids eggs, *Cystoisospora* spp. oocysts and *Giardia* cysts during my research period in 2014

- Taeniids

Taeniids	Dog eating faeces	Dog does not eat faeces	Unknown	Blank	Total
Positive sample	3	1	0	0	4
Negative sample	367	467	1	32	867
Total	370	468	1	32	N = 871

- *Cystoisospora* spp.

<i>Cystoisospora</i> spp.	Dog eating faeces	Dog does not eat faeces	Unknown	Blank	Total
Positive sample	3	4	0	0	7
Negative sample	366	457	1	32	856
Total	369	461	1	32	N = 863

- *Giardia duodenalis*

<i>Giardia duodenalis</i>	Dog eating faeces	Dog does not eat faeces	Unknown	Blank	Total
Positive sample	15	12	0	1	28
Negative sample	355	456	1	31	843
Total	370	468	1	32	N = 871

Appendix D: The statistical analyses performed for the faecal samples examined during my research period in 2014

The statistical analyses were performed using SPSS 22.0 software for Windows:

- *Toxocara* spp.– Pearson Chi-Square & Binomial probability test

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3,998 ^a	1	,046		
Continuity Correction ^b	2,697	1	,101		
Likelihood Ratio	4,006	1	,045		
Fisher's Exact Test				,075	,050
N of Valid Cases	35				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 5,20.

b. Computed only for a 2x2 table

Binomial Test

	Category	N	Observed Prop.	Test Prop.	Exact Sig. (2-tailed)	Point Probability
coprophagy	Group 1	2,00	6	,43	,50	,791
	Group 2	1,00	8	,57		,183
	Total		14	1,00		

- Taeniids – Fisher's Exact Test

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,551 ^a	1	,213		
Continuity Correction ^b	,549	1	,459		
Likelihood Ratio	1,579	1	,209		
Fisher's Exact Test				,326	,230
N of Valid Cases	838				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 1,77.

b. Computed only for a 2x2 table

- *Cystoisospora* spp. – Fisher's Exact Test

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,007 ^a	1	,932		
Continuity Correction ^b	,000	1	1,000		
Likelihood Ratio	,007	1	,932		
Fisher's Exact Test				1,000	,620
N of Valid Cases	830				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 3,11.

b. Computed only for a 2x2 table

- *Giardia duodenalis* – Pearson Chi-Square

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,471 ^a	1	,225		
Continuity Correction ^b	1,032	1	,310		
Likelihood Ratio	1,458	1	,227		
Fisher's Exact Test				,242	,155
N of Valid Cases	838				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 11,92.

b. Computed only for a 2x2 table