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Co-infection of *Giardia duodenalis* with other intestinal parasites in group housed dogs

Relationship between the presence of diarrhea and the prevalence of intestinal parasites

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Abstract

This study has examined whether group housed dogs, which are infected with *Giardia duodenalis* and are having loose stool, are more likely to be infected with other intestinal parasites as well. 344 faecal samples of group housed dogs were examined using the Centrifuge Sedimentation Flotation method, Idexx SNAP® *Giardia* test and Immunofluorescence assay. 86 (25%) samples turned out to be positive of *Giardia duodenalis*, of which 28 (33%) samples were labeled as “diarrhea”. Of the 28 diarrhea samples, 17 (60%) samples were positive for at least one other intestinal parasite. Of the 58 (67%) faecal samples with a normal faeces consistency, 15 (26%) samples were found positive for at least one other parasite. It was concluded that there are signs of a probable positive statistical relationship between the presence of diarrhea in dogs with giardiasis and the presence of at least one other intestinal parasite. However, further research will be needed to specify the strength and interdependence of this relationship, especially since the nature of the research method and sample size leaves room for explanatory validation.

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1 Introduction

Veterinary research has spent much effort on the examination of *Giardia duodenalis* (syn. *G. lamblia, G. intestinalis*). *Giardia duodenalis*, hereafter abbreviated as *G. duodenalis*, is a flagellate protozoan which is, commonly found in the faeces of several mammals. Generally, *G. duodenalis* infections are considered to occur more often in animals living in crowded environments, such as shelters or breeding kennels, than in animals from small breeders or kept individually as pets. Furthermore, younger animals seem to be at higher risk for a *G. duodenalis* infection[1]. Moreover, infected dogs can have signs of diarrhea which is caused by malabsorption, maldigestion or increased motility, but can be asymptomatic as well[1-3].

Previous research on *G. duodenalis* has defined eight assemblages. These assemblages are named on a categorical scale of A-H. To be specific, humans can be infected with Assemblages A and B, whereas dogs will mainly be infected with C and D. In addition, cloven-hoofed animals can be infected with Assemblage E, cats with Assemblage F and Assemblage G can be found in rats[2]. Finally, Assemblage H can be found in gulls and seals[4]. Although dogs are primarily infected with Assemblage C and D, dogs (and also other mammals) can get infected with Assemblage A or B as well[2, 5]. Therefore, *G. duodenalis* found in dogs and cats has to be considered as a potential zoonotic risk[2].

In addition, more in-depth research has been performed on the relationship between the presence of *G. duodenalis* and other intestinal parasites[6-8]. For example, helminths were concluded to have some correlation (both positive and negative) with the presence of *G. duodenalis* in several species, however these findings are not generalizable nor deemed conclusive[8]. This will be further elaborated on later in this chapter.

My research will contribute to existing findings in the field of *G. duodenalis* by further explaining the presence of diarrhea while infected with *G. duodenalis* in relationship to infections by other parasites in a sample of group housed dogs. By means of a structured observation this research is performed to further complement veterinary research on *G. duodenalis*. The main research question is therefore as follows:

Are Giardia duodenalis *infected dogs, who have diarrhea, more likely to be infected with other parasites as well, in comparison with* Giardia duodenalis *infected dogs who do not have diarrhea?*

Before the hypotheses are provided, characteristics of *G. duodenalis* will be further addressed to gain additional understanding of the research topic.

1.1 Lifecycle & transmission of *Giardia duodenalis*

Many researchers have focused their research on the lifecycle of *G. duodenalis*. The life cycle of *G. duodenalis* begins with the excretion of cysts in the faeces of an infected animal. These cysts are infective immediately and environmentally resistant. They contain four nuclei. Once ingested, the cyst excysts in the duodenum and four trophozoites are released, due to the acidic pH in the stomach and the pancreatic enzymes trypsin and chemotripsin[1, 9-11]. The trophozoites contain two nuclei each[1, 9] and remain in the lumen of the intestines, where they replicate by binary fission in the duodenum or upper jejunum[2, 9, 12]. The trophozoites can either be attached to the enteric mucosa by a sucking disk on their ventral side, or free in the lumen.

As the trophozoites transit towards the colon, cyst formation occurs and these cysts will be shedded in the faeces. In cases of excessive trophozoites can also be excreted in the faeces, but they can hardly survive because of a lack of resistance in the environment, in contrast to the cysts[2]. The lifecycle of *G. duodenalis* is illustrated in figure 1.

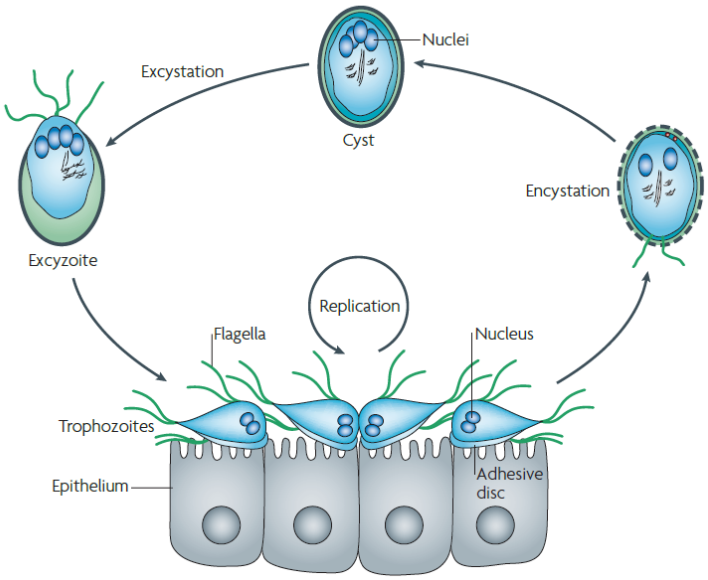

Figure 1. Life cycle of Giardia duodenalis[11]

Because it can be challenging for shelters to keep up hygiene, transmission of *G. duodenalis* by shelter dogs takes most commonly place through ingestion of cysts when consuming water or food that is highly contaminated. Furthermore, infection by *G. duodenalis* can also occur from direct contact with infected dogs[13], coprophagy or contaminated fomites[1]. Moreover, animals in shelters are usually more stressed, which can be of influence on the immune system and therefore can predispose for infection. It has been demonstrated in humans that a few cysts (10-100) can lead to cause an infection[1].

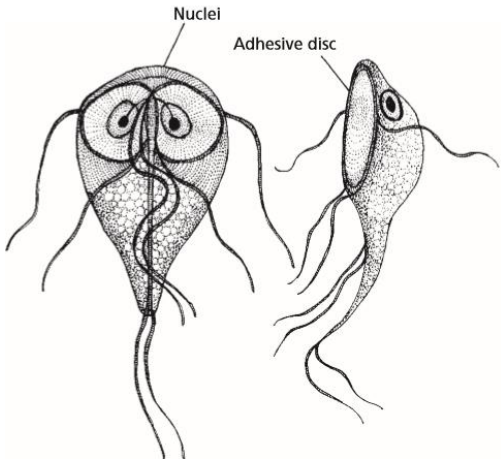

Figure 2. Trophozoite of G. duodenalis [14]

1.1.1 Symptoms

As mentioned before, most cats and dogs infected by *G. duodenalis* remain asymptomatic. When they do show symptoms, it's mostly diarrhea as a result of malabsorption. Studies have reported that giardiasis may lead to damage of the epithelial barrier, villus atrophy, increased permeability of the intestines and apoptosis of the enterocytes. This damage is partly due to the humoral immune response[15].

Normally, the diarrhea is self-limiting in immunocompetent animals. Weight loss may occur in cases of chronic malabsorption[10]. According to Troeger et al (2007) chronic infections of *G. duodenalis* in humans can also result in hypersecretion of chloride[16]. Buret (2007) complements this and mentions the fact that a combination of electrolyte secretion and malabsorption appears to be responsible for the amount of fluid in the intestines, and therefore diarrhea[15].

1.2 Diagnose

To diagnose a *G. duodenalis* infection, fresh stool samples are needed. These stool samples can be examined in different ways, including by Centrifuge Sedimentation Flotation method (CSF)[12], Idexx SNAP® *Giardia* test (ELISA), and immunofluorescent antibody test[2]. Idexx SNAP® *Giardia* test detects antigens, while the Centrifuge Sedimentation Flotation and the Immunofluorescent Antibody test identify the presence of cysts. G. duodenalis is shed intermittent or the secretion of cysts may fluctuate from a few cysts to really high concentrations, increasing the risk of a false-negative result[10]. Due to this fact, stool samples of three following days are needed to diagnose, especially when using the CSF-method or Idexx SNAP® *Giardia* test[3]. More information about these tests is explained in "Material & Methods". If a *G. duodenalis* infection is diagnosed, the next step is to select the most appropriate method of treatment. Though this is not the scope of this report, the next paragraph will briefly highlight the therapy of *G. duodenalis.*

1.3 Therapy

The standard treatment of *G. duodenalis* infection in dogs is the administration of fenbendazole (50mg/kg), once a day during three days[17]. Fenbendazole is an active compound that is administered to treat infections with – amongst others – roundworms (*T. canis, T. leonina*), whipworms (*T. vulpis*), hookworms (*A. caninum*), protozoa (*G. duodenalis*), and certain tapeworms[18]. If the clinical symptoms are still present after three days of treatment with fenbendazole, therapy can be extended with an additional three days. Moreover, other than fenbendazole, metronidazole can be used to treat *G. duodenalis* as well.

Metronidazole is an antibiotic that should be administered twice a day (25 mg/kg) during five consecutive days and is, next to an infection with *G. duodenalis*, also used for treatment against *Clostridium* spp.. Metronidazole may also have possible anti-inflammatory properties[10, 17, 19] Since metronidazole is an antibiotic and is known for its side-effects (neurologic symptoms), fenbendazole is the preferred choice of treatment for *G. duodenalis*. To complement therapy, the medicinal treatment can be supported by a high fiber diet[10].

Apart from medicinal methods used to treat animals with *G. duodenalis*, prevention of infection can be an effective method to avoid animals being infected. In the next paragraph, several ways to prevent a *G. duodenalis* infection are discussed.

1.4 Prevention

Next to medicinal treatment of the animal itself, improving the contingency factors in the environment of the animal is described as a highly important step in treating and/ or preventing infections. First, in order to decrease the probability of *G. duodenalis* being transmitted to other animals, faeces should be removed from the animals living conditions in an adequate manner[10, 17].

Furthermore, contaminated surfaces should be thoroughly cleaned and disinfected. Disinfectants should contain quaternary ammonium compounds, which can inactivate the *G. duodenalis* cysts[10]. Since *G. duodenalis* thrives in a humid environment, it is key to make the living conditions as dry as possible after it has been cleaned. Last, shelter owners should make sure that the animal troughs are cleaned regularly (for instance every day), and they should consider to bathe the infected animals, to get rid of

cysts in their coat[10]. However, where my research is performed in an animal shelter environment, no attention will be paid to the contingency factors where the population is kept.

An important factor in this research is the prevalence of other parasites in dogs that are affected by *G. duodenalis*. In the following part other intestinal parasites are described that are found commonly in the Netherlands.

1.5 Other intestinal parasites

In this chapter, a number of intestinal parasites is described that are commonly found in dogs living in the Netherlands. After a summary is given of these parasites, existing research on the concurrent effect with G. duodenalis is described.

1.5.1 Coccidia

The most important genera of the coccidian-family are *Cystoisospora* spp. (*Isospora*) and *Eimeria*. The difference between both genera is, among other things, decided by the number of sporocysts in each oocyst after sporulation, and the quantity of sporozoites in each sporocyst. Oocysts of *Cystoisospora* consist of two sporocysts each, with four sporozoites per sporocyst. *Eimeria* has four sporocysts per oocyst, and two sporozoites per sporocyst. *Eimeria* is not clinically relevant for dogs, since it infects herbivores and birds and is only found in dog's faeces coincidentally, for example after coprophagy or predation. *Cystoisospora*, however, infects carnivores and omnivores and is therefore clinically relevant for dogs[14].

Cystoisospora canis

Cystoisospora canis can be transmitted through predation of rodents or eating meat from – amongst others – ruminants. Dogs can also ingest sporulated oocysts, which release sporozoites in the intestines and penetrate the epithelial cells. Here, consequently the first schizogony takes place (asexual reproduction) followed by other schizogenic phases or gametogony (sexual reproduction which produces a zygote)[14, 17]. Subsequently, the zygote develops to an oocyst that breaks out of the epithelial cell and leaves the host via the faeces. The prepatent period lasts 8 to 11 days and mainly young animals can shed large numbers of oocysts. With minor infections normally there are no symptoms, where heavy infections can cause diarrhea accompanied by fever, weight loss, dehydration and anorexia. Diagnose can be done by faecal examination[17].

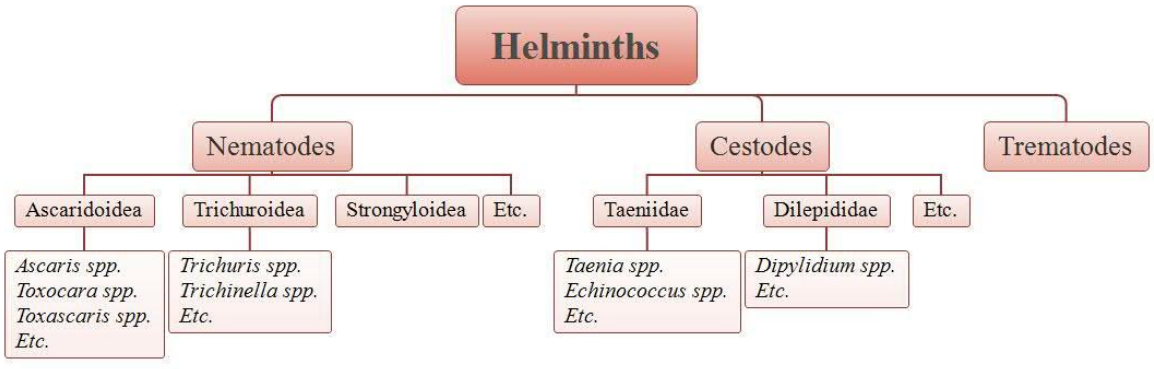

Figure 3. Small part of the taxonomy of helminths[20]

1.5.2 Cestodes

There are many different types of tapeworms, where *Dipylidium caninum* and *Taenia* spp. are the most commonly found in dogs in the Netherlands.

Dipylidium caninum

This tapeworm has an indirect life cycle, with fleas and biting lice as its intermediate hosts. After ingestion by the intermediate host, the eggs develop into cysticercoids in the abdominal cavity[17, 21]. If a dog ingests an infected flea or louse, the adult tapeworm will develop in the small intestines[17, 22]. The adult worm attaches to the intestine wall, causing a local inflammation. The arised laesions are usually minor, therefore most infections are symptomless. In cases of severe infections, gastrointestinal symptoms may be seen, as a result of a catarrhal enteritis, villushypertrophy and small bleedings of the intestinal wall.

The adult tapeworm exists of proglottids, which each contains ten thousands of eggs. These proglottids can detach and leave the host actively via the anus of the host. This causes pruritus and is often noticed when dogs rub their rear ends on the floor[17]. The proglottids can rupture, releasing the eggs into the environment and completing its life cycle[22]. *D. caninum* is considered a zoonotic risk and can be diagnosed by identifying proglottids or eggs in the faeces by a combination of macroscopic and microscopic examination[17, 22].

***Taenia* spp.**

Taenia spp. are large tapeworms with livestock as its intermediate hosts. Dogs get infected by eating infected preys. Like *D. caninum*, dogs get infected by consuming the cysticerci of *Taenia* spp. in the intermediate host, where the cysticerci develop into an adult tapeworm in the small intestines[17, 21]. Infections are mainly asymptomatic. In contrast to proglottids of *D. caninum*, which can leave the host actively via the anus, the proglottids of *Taenia* spp. leave passively with the faeces. Again, diagnosis can be made by a combination of macroscopic and microscopic examination of the faeces[17].

***Echinococcus* spp.**

Echinococcus spp. are rarely found in the Netherlands[17]. *Echinococcus granulosus* is a tapeworm with livestock as its intermediate hosts and canids as its final hosts. *Echinococcus multilocularis* has mostly rodents as its intermediate hosts and foxes and occasionally other carnivores as its final hosts[9, 17]. The infective eggs, containing oncospheres, are ingested by the intermediate host. The oncospheres penetrate the gut wall and travel via the circulatory system to the liver. In the liver, the oncospheres develop during the metacestode stage into a alveolar or multilocular cyst[9]. Next, final hosts get infected by consuming an infected intermediate host. Infections in the final hosts are mostly asymptomatic. Diagnosis is difficult, because the proglottids are small and are shedded intermittently. Examination of faeces can be done for diagnosing, but the eggs can't be differentiated from eggs of *Taenia* spp. using the CSF. PCR or ELISA can be useful for differentiating[9, 17].

1.5.3 Nematodes

1.5.3.1 Ascaridoids

The ascarid nematodes are one of the largest nematodes and can infect most domestic animals. A lot of different ascarid nematodes exist, including *Toxocara canis and Toxascaris leonina*[21].

Toxocara canis

Toxacara canis is an ascarid nematode that is found worldwide. In the Netherlands and Belgium 4,6% of the household dogs[23] and 15-36% of the shelter dogs and stray dogs shed its eggs via the faeces[17, 24]. The eggs embryonate in the environment, where a L3-larvae develops in the egg. In optimal circumstances the development will approximately take between one to two weeks, but most often this will take between four to six weeks before the eggs are infective. These eggs are very resistant and are able to stay infective for many years. Dogs can get infected through several ways, such as via the

placenta, via milk during lactation, through oral ingestion of the eggs or by the predation of an intermediate host[17].

In puppies, the larvae hatch in the small intestines. Thereafter, the larvae migrate through the blood via the liver to the lungs. After the second molting they return via the trachea to the small intestines, where they also molt twice and mature[9]. These adult worms produce eggs that are secreted in the faeces. This is called the hepatic-tracheal transmission. As the pups grow older this type of transmission is gradually replaced by the somatic migration. In that case, the larvae will travel by the bloodstream and enter skeletal muscles and organs like heart, brains and liver, where they encyst[17, 24].

Infected, adult dogs rarely show signs of infection. In mild infections, there is no noticeable damage to the tissues, and therefore the dog shows no symptoms. In severe infections, the larvae can cause pneumonia (during the pulmonary migration) and a mucoid enteritis. Pups, who are heavily infected by *Toxocara canis*, can even die because of the lesions. It is not clear if *Toxocara canis* can cause neurological signs as well[9]. It can be diagnosed by microscopic determination of the eggs in the faeces. However, detecting eggs in dog's faeces does not necessarily mean that the dog is infected. Many dogs show coprophagic behavior, and non-infective eggs can pass passively the gastrointestinal tract, resulting in a false positive diagnosis. This applies for many other intestinal parasites as well[17, 25].

Toxascaris leonina

This ascarid nematode is a parasite of the small intestine but causes little to no symptoms. After ingestion of embryonated eggs, L2 larvae invade the intestinal wall, molt twice and then return to the intestinal lumen for further developing into adult stages[17, 21]. Unlike *Toxocara canis* and other ascarid nematodes, systemic migration does not occur[21, 26].

After shedding into the external environment, it only takes 3 to 10 days for the eggs containing infective second stage larvae[21]. The eggs are highly resistant and can remain infective for up to 3 years. Virtually all warm blooded animals can act as a paratenic host, where the larvae develop to L3 larvae. After uptake of the intermediate host, development of the third stage larvae to the adult stage in the intestines can take 8 weeks.

In general *T. leonina* does not cause any symptoms. Only in case of heavy infections, *T. leonina* can cause a mucoid eosinophilic gastroenteritis, which expresses itself as diarrhea or obstipation. To set the diagnosis of *T. leonina*, faecal examination is necessary[17].

1.5.3.2 Whipworms

Whipworms have a tapered anterior oesophageal end and a thicker and shorter posterior part. There are a lot of different types of whipworms, of which *Trichuris vulpis* is of clinical interest in dogs[21].

Trichuris vulpis

T. vulpis is not commonly found in household dogs (0,7-5%), but 25-29 percent of the shelter dogs is infected by this whipworm[17]. Dogs can get infected by oral ingestion of the embryonated eggs containing L1-larvae. The larvae moult several times and matures after 60-90 days in the wall of the intestines. These worms produce eggs which are secreted in the faeces and are infectious after ten days. These eggs are quite resistant and can survive in the environment for over five years. Most infections are subclinical, but during a severe infection the worms can cause a haemorrhagic colitis, which can lead to watery diarrhea containing blood[9, 17]. This can lead to anaemia and heavily infected pups can even die. Faecal examination can be done to diagnose *T. vulpis*[17].

1.6 Concurrent infections

This chapter gives a brief description of existing research on the interrelationships between *G. duodenalis* and other intestinal parasites.

Research in humans proved that an association exists between a helminth infection and a *G. duodenalis* infection. First, the research of Blackwell et al (2013) showed that individuals that are infected with *G. duodenalis* are less likely to be infected with hookworms. At the same time, there is a lower probability that individuals infected with hookworms are infected with *G. duodenalis*. Second, *G. duodenalis* infection has also been associated with a lower prevalence of *Ascaris lumbricoides* and *Strongyloides stercoralis.* However, *Trichuris trichiura* showed to have a positive relationship with *G. lamblia,* meaning that humans infected with the first were likely to be infected with the latter[8].

Third, Blackwell et al (2013) concluded that the probability of a successful treatment of *G. duodenalis* increased in the presence of a co-infection with helminths. Yet, treating a helminths infection successfully deemed less probable when a *G. duodenalis* infection was present. When an individual was treated for an helminth infection, that person became more sensitive for a *G. duodenalis* infection[8].

A similar finding was done in the research of Bajer et al (2011). In this research, sled dogs in Poland were examined for the prevalence of intestinal parasites, which pointed out a negative correlation between the presence of *G. duodenalis* and the presence of nematodes. The *Giardia* parasite occurred twice as often in dogs that did not carry a nematode infection, in comparison with dogs that did suffer an infection with nematodes[7].

Another contribution in the field of *G. duodenalis* by Von Allmen et al (2006) with mice, however, showed a different conclusion. In this research a concurrent infection of the nematode *Trichinella spiralis* and *G. duodenalis* was observed which showed that an infection of *T. spiralis* actually stimulated the growth of trophozoites of *G. duodenalis*. In addition, the research results demonstrated that mice, suffering from an intestinal *T. spiralis* infection, were more sensitive for a *G. duodenalis* infection. The severity and duration of the primary *G. duodenalis* infection increased when mice got an infection with *T. spiralis* as well[6].

Summarizing, a *G. duodenalis* infection in either animals or humans are found to have different correlations with the presence of other intestinal parasites. In this study with group housed dogs, it was examined if signs could be found of a correlation between other intestinal parasites and the presence of diarrhea in dogs with giardiasis.

1.7 Field Research

Research on the relationship between an infection with *G. duodenalis* and other intestinal parasites has shown to have different results. During this research the relationship between the faecal consistency and co-infection with *G. duodenalis* and other intestinal parasites is examined. Therefore, the following main question was defined:

Are Giardia duodenalis *infected dogs, who have diarrhea, more likely to be infected with other intestinal parasites as well, in comparison with* Giardia duodenalis *infected dogs who do not have diarrhea?*

Based on this main question, the next hypotheses will be tested:

H_0: *G. duodenalis* infected dogs, who have diarrhea, are not more likely to be infected with at least one other intestinal parasite as well.

H_1: *G. duodenalis* infected dogs, who have diarrhea, are more likely to be infected with at least one other intestinal parasite as well.

2 Material and Methods

Eighteen different animal shelters and kennels, situated in different regions in the Netherlands, were visited between October 2013 and May 2014. 344 singular faecal samples were collected from dogs who were required to be older than six months and had to be living uninterruptedly in the kennel or shelter. The purposes of the kennels ranged from housing stray dogs and abandoned dogs, housing hunting dogs, etcetera.

2.1 Collecting faecal samples

The collected faecal samples had to be less than two days old and were put individually in plastic bags and plastic containers providing the sample with an unique code, so the samples would not get mixed up and it would be clear which sample belonged to which dog. The samples of the two hunting dog kennels are unknown to which dog they belong. The hunting dogs were housed in big groups (50-60 dogs) and the faeces was all over the place, so it is possible those samples have been mixed. The faecal samples were stored at 4°C until examination (for maximal two days). If possible, from each dog, information was provided about the age, gender, neuter status and breed of that dog, whether the dog was showing signs of giardiosis (diarrhea/ emaciation), treatment, date of entrance to the kennel and the section of the shelter the dog was living. In the animal shelters, it was also asked if the dog was a stray dog or abandoned by its owner.
Also, a survey was done, to gain some information about among other things the policy of deworming and cleaning and the knowledge of the co-workers of the kennel about *G. duodenalis*. The result and conclusion of this survey is available in the research paper of Anouk Overbeek.

2.2 Examination of the faecal samples

The faecal examination was performed with three different tests; Centrifugation Sedimentation Flotation (CSF), Idexx SNAP® *Giardia* test and direct immunofluorescence assay Merifluor® *Giardia/ Cryptosporidium* test (IFA). In the beginning of this study, the Idexx SNAP® *Giardia* test was not available yet. Because of this, the faecal samples were stored at -20 °C after examination with the CSF. When the Idexx SNAP® *Giardia* test became available, these frozen faecal samples could be thawed and used for examination with Idexx SNAP® *Giardia* test. To keep the circumstances as similar as possible, this has been done during the whole study, even when Idexx SNAP® *Giardia* test was available. At first, the fresh faecal samples were examined macroscopically and graded for consistency. The grading system for faeces consistency can be found in Table 1 below. Faeces with a consistency < 3 was classified as diarrhea,

Table 1. Grading system of faeces consistency

Grade	Term	Description
1	Aqueous faeces	Watery faeces that flows like liquid
2	Thin faeces	Liquid faeces, but not flowing
3	Soft faeces	Ordinary looking, of which the shape alters after being touched gently
4	Firm faeces	Solid faeces, of which the shape does not alter after being picked up
5	Hard faeces	Fragile, but of which the shape does not alter after being touched firmly
6	Very hard faeces	Very hard faeces, which is almost unbreakable
7	Friable faeces	Crumbling faeces, which falls apart after being touched

2.2.1 Centrifuge Sedimentation Flotation

A suspension was made with 3-5 gram of faecal material in approximately 55 ml water, using a mortar. Sometimes faecal samples were pooled by combining two samples in the suspension, so more samples could be examined in one day. Pooled faecal samples were suspended in approximately 110 ml water. This suspension was sieved to remove debris and poured in a centrifuge tube. This was centrifuged at 3000 rpm, during 2 minutes. Next, the supernatant was poured off and a small amount of sucrose solution (with a specific gravity of 1.30 $g.cm^{-3)}$ was added to the remaining sediment in the centrifuge tube. By vortexing, the sediment got resuspended in the sucrose solution. Thereafter, more sucrose solution was poured in the centrifuge tube to finally form a positive meniscus. A coverslip was then placed on the meniscus and centrifuged at 3000 rpm for 2 minutes to concentrate any parasite eggs, cysts or oocysts in the upper layer. Next, the coverslips were removed perpendicular from the meniscus and placed on a microscope slide. The entire coverslip was examined for eggs and oocysts at 100 x magnification. Subsequently, the microscope slide was examined 2-3 rows at a magnification of 250 or 400 for detection of *G. duodenalis* cysts. A faecal sample was considered positive if one or more *G. duodenalis* cysts, helminth eggs or oocysts were identified in the microscope slide. The cyst were also determined semi-quantitatively. If a pooled sample tested positive for *G. duodenalis*, both samples were examined individually using the CSF, to determine which dog was positive for *G. duodenalis* cysts.
The entire protocol used can be found in Appendix B.

2.2.2 Immunofluorescence assay

1-2 gram of faecal material was suspended in sodium acetate-acetic acid-formalin (SAF) and strained to withhold large debris. This suspension was put in a centrifuge tube and centrifuged at 3000 rpm for 5 minutes. The supernatant was decanted and the sediment was resuspended by adding aquadest up to a volume of 1 ml. After vortexing, a transferloop was used to apply a drop of this suspension on a IFA slide, which had to dry for 30 minutes at room temperature. Next, successively a drop Detection reagent and a drop Counterstain were applied. This was mixed by a glass stick and incubated in a humified chamber for 30 minutes. After incubation, the excess of Detection Reagent and Counterstain was removed by rinsing the slide with Wash Buffer. Finally, a drop of Mounting medium was applied and the slide was covered by a cover slide.
The IFA slide was examined by a fluorescence microscope at a 100x magnification, and a 400x magnification was used for further determination. Detection of one *G. duodenalis* cyst signified a positive faecal sample. The complete protocol used can be found in Appendix C.

2.2.3 Idexx SNAP® *Giardia* test

The Idexx SNAP® *Giardia* test can be used to detect *G. duodenalis* antigen in faeces from dogs and cats. This test was performed as instructed by the manufacturer. After thawing the faecal samples to room temperature, the swab tip of the conjugate/swab device was coated with a thin layer of faeces and replaced in the tube. The conjugate solution was then released into the tube to the swab tip. The swab was then used as a pipette and 5 drops of sample/conjugate solution were placed into the sample well of the SNAP device. The test result could be read after 8 minutes. A faecal sample was scored as positive if the sample on the *G. duodenalis* sample spot was darker than the color on the negative control spot. The entire protocol used can be found in Appendix D.

2.3 Statistical analysis

The data obtained from the field research in the kennel is analyzed using the statistical program SPSS. After importing the data, the data was checked for outliers. In this dataset no outliers were found. Furthermore since the data is categorical (“0” or “1”) no missing values were recoded. However, the questions were required to be recoded because SPSS cannot analyze 0 values and the data was entered as either “0” or “1”. Therefore the values for questions as “*G. duodenalis* infected?” and “Other intestinal parasites infected?” were recoded; “0” to “No” and “1” to “Yes”. In addition, the category “Faeces consistency” was recoded to “Diarrhea?” with values “<3” to “Yes” and “>2,5” to “No”.

The data collected is referred to as categorical data, which is, according to Saunders et al (2009)**,** defined as data of which the values cannot be measured numerically. This type of data can either be classified into categories on the basis of the characteristics - that identify or describe the variable - or placed in rank order. Furthermore, this data can be placed in the descriptive data category, since it is not possible to define the data numerically or to rank it[27]. In the following chapter, the hypothesis as defined in chapter 1.7 will be further analyzed using SPSS.

In order to find a relationship between two categorical variables generally three steps are taken. First, statistical analysis is required to determine if there is a relationship. For testing on relationships between nominal variables the Pearson Chi Square Test will be used. The Pearson Chi Square test is allowed when less than 20% of the cells in the cross tabulation have an expected count of less than 5. In case these conditions are not met, the Fisher's Exact Test will be used to determine if there is a statistical relationship between variables.

Second, the strength of the relationship is determined by using the association size *Phi*. This test is generally used for 2x2 tables and is therefore applicable for testing the strength of a relationship between categorical variables "Diarrhea' and "Infection of other intestinal parasites".

Third, an assumption is made whether the relationship is positive or negative using the Odds Ratio. In the next chapter the results of these tests are provided.

3 Results

3.1 Hypothesis testing

To test the hypothesis posed in chapter 1.7, a Pearson Chi-Square (X^2) test was conducted to measure if a statistical relationship exists between the variables "Diarrhea" and the variable "Infection of other intestinal parasites" within *G. duodenalis* infected dogs. The Chi-Square is allowed because the data is categorical and therefore on a nominal scale.

With the Chi-Square test for every value the expected count *E* is compared to the observed count *O*. The Chi-Square test is a measurement if a statistical relationship exists, afterwards the phi coefficient is used to measure the strength of the correlation.

H_1: *G. duodenalis* infected dogs, who have diarrhea, are more likely to be infected with at least one other intestinal parasite as well

This hypothesis is tested to conclude if there is a relationship between the presence of diarrhea in *G. duodenalis* infected dogs and at least one other intestinal parasite. When the Chi-Square test is used as a test of independence, the Chi-Square test must be applied to cross tabs. The cross tabulation and Chi-Square test results for hypothesis H_1 are shown below.

Table 2. Crosstabulation; G. duodenalis *infected dogs with diarrhea x infected with at least one other intestinal parasite*

Diarrhea? * Infected with other parasites? Crosstabulation

			Infected with other parasites?		
			No	Yes	Total
Diarrhea?	Yes	Count	11	17	28
		Expected Count	17,6	10,4	28,0
	No	Count	43	15	58
		Expected Count	36,4	21,6	58,0
Total		Count	54	32	86
		Expected Count	54,0	32,0	86,0

Table 3. Statistical analysis of G. duodenalis *infected dogs with diarrhea x infection with at least one other parasite*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	9,818 [a]	1	,002		
Continuity Correction [b]	8,382	1	,004		
Likelihood Ratio	9,703	1	,002		
Fisher's Exact Test				,004	,002
N of Valid Cases	86				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 10,42.

b. Computed only for a 2x2 table

As can be seen here, 0 cells (0,0%) have an expected count of less than 5 and all the values in the cells are larger than 1. This means that all conditions are met for using the Chi-Square test to test for a statistical relationship between the variables. This means that the conditions for the Chi-Square test are appropriate to test for the probability of a statistical relationship between these variables.

The Pearson Chi-Square shows a $X^2(1)$ value of 9,818 and p = 0.002. The p-value is therefore lower than $\alpha = 0,05$, which means that it is reasonable to reject H_0 and to assume that there is a probability that the variables “Diarrhea” and “Infection of other intestinal parasites” have a statistical relationship with a reliability of 95%. Hereafter, the phi coefficient has been used to test for the strength of the relationship and gives a value of 0,338, which means a medium strength (0,3< value < 0,5) of the relationship.

Table 4. Phi coefficient

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	,338	,002
	Cramer's V	,338	,002
N of Valid Cases		86	

To determine whether this relationship is positive or negative, the odds ratio has been computed by using the formula $OR = \frac{a*d}{b*c}$. Calculating the Odds Ratio gave a value of 4,43. This means that the presence of diarrhea in *G. duodenalis* infected dogs raises the odds of the presence of other intestinal parasites as well. The OR is significant since the value 1 is not within the 95% confidence interval.

Table 5. Odds Ratio

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Diarrhea? (No / Yes)	4,430	1,697	11,565
For cohort Infected with other parasites? = No	1,887	1,162	3,065
For cohort Infected with other parasites? = Yes	,426	,251	,722
N of Valid Cases	86		

3.2 Testing for individual parasites

To specify these results, the tests as mentioned above are performed for each individual intestinal parasite. An overview of the test results can be found in Table 6 below.

First, it was checked if the conditions for using the Pearson Chi-Square test were met. It turned out that for determining the relationship between the presence of diarrhea and the intestinal parasites *T. canis*, *T. leonina*, *C. canis* and *Capillaria* the conditions were not valid since at least 20% of the cells did have an expected count less than 5. Therefore, for these relationships the Fisher's Exact Test was applied. For the other relationships it was found that the Pearson Chi-Square was applicable.

The Fisher's Exact Test for the relationship between the variables "Diarrhea" and "*T. canis"* shows a 1-sided value of 1,000. This value is significantly higher than α = 0,05 and therefore there is no reason to assume a statistical relationship between these variables. The Fisher's Exact Test for the variables "Diarrhea" and either "*C. canis*" or "*Capillaria*" shows a p-value of respectively 0,246 and 0,548 (higher than α = 0,05) and therefore there is no reason to assume a statistical relationship.

For the relationship between diarrhea and *T. Leonina* the Fisher's Exact test shows a value of 0,036 (lower than α = 0,05) and therefore there is reason to assume a statistical relationship between these variables.

The Pearson Chi-Square test shows a p-value lower than α = 0,05 for the intestinal parasites *T. vulpis* (0,001) and possible hookworms (0,000). This means that that there is a possible statistical relationship between the variables "Diarrhea" and "Individual intestinal parasite", whereby "Individual intestinal parasite" is equal to *T. vulpis* and possible hookworms.

Next, in order to determine the strength of the relationship, the phi coefficient has been determined. This gives the following values: *T. vulpis* ($\varphi = 0{,}365$), possible hookworms ($\varphi = 0{,}385$) and *T. leonina* ($\varphi = 0{,}231$). A medium strength is found between diarrhea on the one hand and the intestinal parasites *T. vulpis* and possible hookworms on the other hand. The relationship between "diarrhea" and "*T. leonina*" is found to be weak.

The crosstabulations, Pearson Chi-Square test results and the Fisher's Exact test results of the individual intestinal parasites can be found in Appendix E.

Table 6. Prevalence and p-value of the individual parasites

	T. canis **+**	***T. canis*** **-**	***T. leonina*** **+**	***T. leonina*** **-**	***T. vulpis*** **+**	***T. vulpis*** **-**	***Taenia* spp./ *Echinococcus*** **+**	***Taenia* spp./ *Echinococcus*** **-**	***C. canis*** **+**	***C. canis*** **-**	**Hookworms +**	**Hookworms -**	***Capillaria*** **+**	***Capillaria*** **-**
Diarrhea +	0	28	8	20	16	12	0	28	2	26	16	12	1	27
Diarrhea -	2	56	6	52	12	46	0	58	1	57	11	47	1	57
P-value	-		-		0,001		-		-		0,000		-	
φ			0,231		0,365						0,385			
Fisher's Exact Test 2-sided sign.	1,000		0,058						0,246				0,548	
Fisher's Exact Test 1-sided sign.	0,452		0,036						0,246				0,548	

4 Discussion and Conclusion

This study has examined if group housed dogs, that are infected with *Giardia duodenalis,* (a) show clinical symptoms (i.e. diarrhea) and (b) are more likely to be infected with at least one other intestinal parasite as well. Existing veterinary research and aligning studies on humans in the field of intestinal parasites have made several assumptions on a correlation between an infection of *G. duodenalis* and the co-existence of other intestinal parasites[6-8].

In this research, different methods of faecal examinations were used for following purpose. The CSF was used for determining all intestinal parasites, including *G. duodenalis.* However, according to Geurden et al (2008), IFA is a more specific and sensitive technique for detecting cysts of *G. duodenalis* in one-day stool samples than ELISA and CSF, and therefore the golden standard[3]. Since one-day stool samples were examined for this research, IFA was considered the most reliable method for diagnosing *G. duodenalis*; faecal samples were considered positive when demonstrated positive by IFA, regardless of the outcome of the other diagnostic tests. This turned out to be 86 out of 344 faecal samples.

After (statistical) analysis of these samples, this research demonstrated that there is a probability of a positive relationship between the presence of diarrhea in *G. duodenalis* infected (group housed) dogs and infections with other intestinal parasites (i.e. *Toxocara canis, Toxascaris leonina, Trichuris vulpis, Taenia* spp*/ Echinococcus* spp.*, Cystoisospora canis,* hookworms and *Capillaria*), taking the limitations of the statistical analysis and this method of research into account. As can be seen in table 2 in chapter 3.1, when the number of infections with other parasites increases this also raises the odds of the presence of diarrhea.

The hypothesis (H_1) assumed a relationship between the presence of diarrhea in *G. duodenalis* infected dogs and the presence of other intestinal parasites. Yet, it showed that this relationship could mainly be influenced by the intestinal parasites *Trichuris vulpis* and possible hookworms. These findings will be discussed next.

4.1 Statistical relationships

The answer on the main research question of this research will be discussed in this paragraph.

Veterinary research in the field of giardiasis, complemented with research on humans, have emphasized that there have been found associations between *G. duodenalis* infections and infections with other infections, but that this is different per intestinal parasite. For example, both veterinary and humane studies demonstrate a negative association between de prevalence of *G. duodenalis* and the prevalence of several helminths[7, 8]. However, it has also been argued that there could be a positive relationship between the presence of *G. duodenalis* and the prevalence of *Trichuris trichiura*[8].

This field study contributes to this existing body of theory by showing that there might be a statistical relationship between the presence of diarrhea in *G. duodenalis* infected dogs and the co-existence of other intestinal parasites. The findings in chapter 3 indicate that there is a chance that at least one other intestinal parasite can influence in some way the infection with *G. duodenalis,* or the other way around. Yet, the same results incline to make an argument that the relationship between the presence of diarrhea in *G. duodenalis* infected dogs and other intestinal parasites is possibly caused in this research population by the prevalence of, specifically, the intestinal parasites *Trichuris vulpis,* hookworms and to a lesser extent *Toxascaris leonina.*

First, the findings indicate that there is a reason to believe that an infection by *Trichuris vulpis* or possible hookworms and the presence of diarrhea in *Giardia* infected dogs have a relationship. The Chi-Square

test on both variables gave a p-value of respectively p = 0,001 and p = 0,000 and since these values are both lower than φ= 0,05, an assumption can be made that there is anything other than chance that the presence of diarrhea in *G. duodenalis* infected dogs on one hand and *Trichuris vulpis* and hookworms on the other hand influence each other. However, when the phi coefficient is observed, it is found that the relationship is not considered to be fierce.

Moreover, *Toxascaris leonina* is found to have some relationship, but the result on this test is, with a p-value of 0,036, less strong and therefore more unlikely. In addition, a reason to assume that there is a statistical relationship between *Cystoisospora canis* or *Capillaria* and the presence of diarrhea in dogs with giardiasis has not been found. Moreover, within *G. duodenalis* infected dogs the tapeworm *Taenia* spp. (or *Echinococcus* spp.) was never observed. This could probably mean that there is a negative association between de prevalence of *G. duodenalis* and the prevalence of *Taenia* spp./ *Echinococcus* spp., but further research would be needed to conclude this.

To nuance the results of this research, it must be stated that p-values are sensitive to sample size and the Chi-Square test does not give much information on the substantive significant in this population. The size of the calculated Chi-Square is directly proportional to the size of the sample, independent of the strength of the relationship between the variables.

4.2 Conclusion

The main research question was formulated as follows:

Are Giardia duodenalis infected dogs, who have diarrhea, more likely to be infected with other intestinal parasites as well, in comparison with Giardia duodenalis infected dogs who do not have diarrhea?

In order to test this main research question, the following hypotheses were formulated:

H_0: *G. duodenalis* infected dogs, who have diarrhea, are not more likely to be infected with at least one other intestinal parasite as well.

H_1: *G. duodenalis* infected dogs, who have diarrhea, are more likely to be infected with at least one other intestinal parasite as well.

Based on the findings discussed in chapter 3, it can be said that there is a 95% probability that an alternative hypothesis for H_0 can be introduced, meaning the probability of a positive statistical relationship between the presence of diarrhea in *G. duodenalis* infected dogs and the co-existence of at least one other intestinal parasite infection. This implies that there is a reason to assume that an infection with an intestinal parasite other than *G. duodenalis* does influence an infection with *G. duodenalis* and diarrhea or the other way around.

Numerous suggestions have been provided for the rational of a positive or negative relationship between concurrent parasite infections. First, an immune response can be of influence on the prevalence of a concurrent infection with another parasite. In some cases a negative association is caused by cross-immunity, whereby it is more difficult for a second parasite to cause an infection if another infection by parasites is already in place. However, the immune response can also create a positive outcome for a second parasite. When the immune system is already triggered by the first infection, it cannot handle a second one whereby other parasites have space to cause an infection as well[6, 8]. Also the location of the infection can play a significant role here. For example, *G. duodenalis* occurs more often in the small intestine, where *Trichuris* occurs more often in the colon[8].

Yet, a number of limitations can be noted that provides room for future research in this area.

4.3 Limitations and further research

Several limitations are applicable to this study. While the study provides contributions to prior research and practice, it also includes downsides. First, in some kennels, the faecal samples were found quite closely next to each other. Therefore it is possible that some faecal samples were constituted of faeces of more than one dog, which could have influenced the results.

Second, the circumstances were different in each kennel, which were not taken into account. It is possible that some dogs were recently dewormed before faecal examination, while for other dogs, it could have been months since the last deworming. Furthermore, each kennel uses other deworming medicine and has its own protocol for cleaning kennels. Also, the dogs were living in different areas of the Netherlands, which could also be a reason of differences in the prevalence of intestinal parasites between the dogs. This could all be of influence of the results.

Third, *G. duodenalis is* secreted intermittently, and therefore it is preferred to use three-day faecal samples. Since it was not possible to use three-day samples, for this research one-day faecal samples were taken, increasing the risk of false-negative results.

This research showed that there is reason to believe that there is a possible positive association between the presence of diarrhea in *G. duodenalis* infected dogs and the prevalence of other intestinal parasites. However, it is not clear yet what the dependent or independent variable is, so whether the presence of intestinal parasites influences the clinical symptoms in *G. duodenalis* infected dogs or the presence of diarrhea in *G. duodenalis* infected dogs influences the presence of other intestinal parasites.

Furthermore, a small group of *G. duodenalis* infected dogs (86) was available, which caused that it was not possible to examine the relationship with the individual parasites apart and it was necessary to take them all together.

The aforementioned limitations imply some recommendations for further research, such as larger research groups and contingency factors.

5 Appendix

A. Requested data for each individual dog

The following questions were asked to provide data for each individual dog and the living conditions of these dogs. This could only be done in the shelters, it was not possible to provide this data for each individual hunting dog. The results are discussed in the research paper written by Anouk Overbeek.

Data per dog

- Name/number of the dog
- Breed
- Gender (male/female)
- Age
- The presence of clinical symptoms of giardiasis (weight loss, diarrhoea)
- Actual treatments of the dog
- Date of entrance in the animal shelter
- Origin of the animal (abandoned by its owner/ stray)
- Section within the shelter where the dog is living in

Survey about the housing

1. How many dogs are housed in the animal shelter/kennel?
2. What is the group size of dogs in the kennel? (individual housing or in groups)
3. What is the policy regarding walking dogs?
4. How does the kennel look (material used, interior etc.)
5. Do all dogs have a free entrance to an outdoor kennel? If yes, what does it look like? (grass, soil, etcetera)
6. Where there any infections with gastrointestinal parasites in the last three months? (if yes, which parasites?)
7. Hygiene policy and protocol used for cleaning and disinfection of the housing facilities:
    a. Frequency of removal of faeces (also from the outdoor playing-fields)
    b. Which detergents and disinfectants are used?
    c. Frequency of cleaning and disinfection
8. Deworming policy:
    a. Which anthelminthic treatments are used?
    b. When are they used? (at entrance?)
    c. Frequency
9. Are there other animal species housed at the animal shelter? Which species?
10. How many puppies are present in the animal shelter and are they housed separated from the adult dogs??

B. Protocol Centrifuge Sedimentation Flotation (CSF) technique

Objective

Quality determination of presence of parasites eggs or (oo)cysts in faeces

Equipment and materials

- Weighing scale (at least accuracy of 0.1 g) (optional)
- Spatula or teaspoon
- Mortar and pestle
- Tea strainer (a coarse sieve)
- A 100 ml beaker (glass or plastic)
- Centrifuge (with tube holders for ca. 12 ml centrifuge tubes; 1500 g which involves ca. 3000 radiations.min^{-1} at a radius of 15 cm)
- Grinded 12 ml centrifuge tubes
- Vortex
- Sucrose solution (specific gravity of 1.30 g.cm^{-3}; dissolve 1280 gram sugar in 1 liter water and check density by weighing 10 ml, which should be 13.0 gram) in a siphon
- Object glasses (microscope slides) and cover slides
- Microscope with ocular 10x and objectives of 4x, 10x and 40x magnification

Procedure

1. Homogenise 3-5 gram (or a teaspoonful) of faeces in ca. 50 ml water using mortar and pestle.
2. Pour faecal suspension over the tea strainer into the beaker to withhold any large debris and homogenize the suspension as the eggs and (oo)cysts will sink.
3. Fill two centrifuge tubes up to ca. 0.5 cm from the top with the filtrate. If an even number of faeces samples are examined, one tube per sample is enough (see next step).
4. Put tubes in the centrifuge. Make sure the centrifuge is in balance by placing two tubes with the same amount of fluid across each other. Centrifuge for 2 minutes at 3000 rpm.
5. Discard the supernatant by pouring it out of the tubes, leaving the pellet with the eggs and (oo)cysts. If the pellet is loose, use a water pump to suck off the supernatant.
6. Fill half the tube with sugar solution and suspend the pellet thoroughly (use vortex).
7. Add more sugar solution up to slightly above the edge of the tube creating a convex meniscus. Either do this before or after putting the tubes back in the centrifuge. If possible, before is preferable and easier.
8. Put a cover slide on top of the tubes and tap lightly with a fingernail.
9. Centrifuge again for 2 min at 3000 rpm.
10. Remove cover slides vertically and put on object glasses.
11. Examine the slides systematically under the microscope at 100x-400x magnification. Use 100x for eggs from most worm species and 400 x for small protozoan cysts or for viewing eggs in more detail.
12. Clean all equipment with (warm) water and if necessary liquid soap as the sugar solution is sticky.

Detection limit with examining one centrifuge tube is (theoretically) ca. 1-2 eggs or oocyst per gram faeces.

Note 1: it takes time to 1280 gram sucrose in 1 liter water. Use hot water and shake or rock frequently until everything is dissolved.

C. Protocol IFA Merifluor® *Giardia/ Cryptosporidium*

This protocol is partially based on the protocol used by Geurden et al (2008)[3]. The faecal material (1-2 g) was preserved in 10% Sodium acetate-acetic acid-formalin solution (SAF) for storage.

Preparation

1. Bring the Merifluor® *Cryptosporidium/Giardia* kit to room temperature before use.
2. Prepare wash buffer: 5 ml wash buffer + 95 ml aquadest.

Procedure

1. Resuspend the suspension by vortexing it thoroughly.
2. Strain the suspension using a tea strainer in order to withhold large debris.
3. Swirl the sieved suspension and collect it into a centrifuge tube.
4. Centrifugation at 3000 rpm for 5 minutes
5. Pour the supernatant off
6. Use aquadest to fill up to a total volume of 1 ml.
7. Resuspend by use of a vortex
8. Use a transfer loop to transfer a drop of faecal material to the treated IFA slide well. Spread the specimen over the entire well. Do not scratch the treated surface of the slide.
9. Also use new transfer loops to transfer a drop of positive and negative control to a separate treated slide well and repeat the procedure described above.
10. Dry the slides at room temperature. This usually requires 30 minutes.
11. Place one drop of Detection Reagent in each well.
12. Place one drop of Counterstain in each well.
13. Mix the reagents with a glass applicator stick and spread over the entire well. Do not scratch the treated surface of the slide.
14. Incubate the slides at 30ºC in a dark, humidified chamber for 30 minutes. Protect the slides from light.
15. Rinse the slides with a gentle stream of wash buffer (5 ml wash buffer diluted in 95 ml aquadest) until the Detection Reagent and Counterstain are removed. Avoid disturbing the specimen or causing cross contamination of the specimens.
16. The excess of the buffer can be removed by tapping the slide on a clean paper towel.
17. Add one drop of Mounting Medium to each well and apply the cover slide
18. Examine each well using 100 x magnification under a fluorescence microscope. This magnification is sufficient for the detection of *Giardia* cysts present.
19. The *Giardia* cyst wall will stain bright apple green. The sample is considered positive when at least one *Giardia* cyst is found on the slide.

D. Protocol used for Idexx SNAP® *Giardia*

This test was performed as instructed by the manufacturer.

Preparation

1. The faecal samples were stored at -20˚C and had to be thawed to room temperature before use.
2. All components of the kit must be at room temperature before use

Test procedure

1. Pull off the tube that covers the conjugate/swab device and coat the entire swab tip with a thin layer of faecal material. Replace the tube over the swab.
2. Break the plastic valve stem inside the reagent bulb by bending the assembly back-and-forth at the neck. Holding the device swab-tip-down, squeeze and release the bulb three times to pass the conjugate solution in the bulb to the swab tip.
3. Place the SNAP device on a flat surface. Remove the tube from the conjugate/swab device. Using the swab/bulb as a pipette, dispense **5 drops of the sample/conjugate solution** into the sample well of the SNAP device, being careful not to splash the contents outside of the sample well. The sample will flow across the result window, reaching the activation circle in approximately 30–60 seconds. Some sample may remain in the sample well.

   When the sample <u>FIRST</u> appears in activation circle, push the activator button firmly until it is flush with the device body

   NOTE: Some samples may not flow to the activation circle within 60 seconds and, therefore, the circle may not turn colour. In this case, press the activator button after the sample has flowed across the result window.
4. Wait **8 minutes**, then read the test result.
   NOTE: The positive control spot colour may develop sooner, but test results are not complete until 8 minutes.

Interpreting Test Results

To determine the test result, read the reaction spots in the result window and compare the colour intensity of the sample spot to that of the negative control spot.

Negative Result

The result is negative for a sample spot, if:

- There is no colour on the sample spot and the negative control spot, *or*
- Colour on the sample spot is equal to the colour on the negative control spot.

Positive Result

Colour on the *Giardia* sample spot is darker than the colour on the negative control spot.

NOTE: Some positive results may have only light colour intensity on the sample spot.

Invalid Result

The negative control spot serves as a safeguard against false-positives. Colour development on the positive control spot indicates the test reagents are functional and helps indicate that the assay has been run properly.

- If the positive control spot does not develop colour, the result is invalid. Repeat the test.
- If colour on the negative control spot is darker than colour on the sample spot, the test is invalid. Repeat the test.

E. Test results individual parasites

Toxocara canis

Table 7. Crosstabulation; G. duodenalis *infected dogs with diarrhea x infected with* T. canis

Diarrhea? * Toxocara canis infected? Crosstabulation

			Toxocara canis infected?		
			No	Yes	Total
Diarrhea?	Yes	Count	28	0	28
		Expected Count	27,3	,7	28,0
	No	Count	56	2	58
		Expected Count	56,7	1,3	58,0
Total		Count	84	2	86
		Expected Count	84,0	2,0	86,0

Table 8. Statistical analysis of G. duodenalis *infected dogs with diarrhea x infection with* Toxocara canis

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,989 [a]	1	,320		
Continuity Correction [b]	,053	1	,817		
Likelihood Ratio	1,599	1	,206		
Fisher's Exact Test				1,000	,452
N of Valid Cases	86				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is ,65.

b. Computed only for a 2x2 table

Toxascaris leonina

Table 9. Crosstabulation; G. duodenalis *infected dogs with diarrhea x infected with* T. leonina

Diarrhea? * Toxascaris leonina infected? Crosstabulation

			Toxascaris leonina infected?		
			No	Yes	Total
Diarrhea?	Yes	Count	20	8	28
		Expected Count	23,4	4,6	28,0
	No	Count	52	6	58
		Expected Count	48,6	9,4	58,0
Total		Count	72	14	86
		Expected Count	72,0	14,0	86,0

Table 10. Statistical analysis of G. duodenalis *infected dogs with diarrhea x infection with* Toxascaris leonina

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4,603 [a]	1	,032		
Continuity Correction [b]	3,363	1	,067		
Likelihood Ratio	4,330	1	,037		
Fisher's Exact Test				,058	,036
N of Valid Cases	86				

a. 1 cells (25,0%) have expected count less than 5. The minimum expected count is 4,56.

b. Computed only for a 2x2 table

*Table 11. Phi coefficient (*T. leonina*)*

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	,231	,032
	Cramer's V	,231	,032
N of Valid Cases		86	

Trichuris vulpis

Table 12. Crosstabulation; G. duodenalis *infected dogs with diarrhea x infected with* Trichurus vulpis

Diarrhea? * Trichuris vulpis infected? Crosstabulation

			Trichuris vulpis infected?		
			No	Yes	Total
Diarrhea?	Yes	Count	12	16	28
		Expected Count	18,9	9,1	28,0
	No	Count	46	12	58
		Expected Count	39,1	18,9	58,0
Total		Count	58	28	86
		Expected Count	58,0	28,0	86,0

Table 13. Statistical analysis of G. duodenalis *infected dogs with diarrhea x infection with* Trichuris vulpis

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	11,428 [a]	1	,001		
Continuity Correction [b]	9,828	1	,002		
Likelihood Ratio	11,151	1	,001		
Fisher's Exact Test				,001	,001
N of Valid Cases	86				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 9,12.

b. Computed only for a 2x2 table

*Table 14. Phi coefficient (*T. vulpis*)*

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	,365	,001
	Cramer's V	,365	,001
N of Valid Cases		86	

Taenia spp./ *Echinococcus* spp

Table 15. Crosstabulation; G. duodenalis *infected dogs x infected with* Taenia *spp.*/ Echinococcus *spp.*

Crosstab

Count

		Taenia spp. / Echinococcus spp. infected?	
		No	Total
Diarrhea?	Yes	28	28
	No	58	58
Total		86	86

Cystoisospora canis

Table 16. Crosstabulation; G. duodenalis *infected dogs with diarrhea x infected with* Cystoisospora canis

Diarrhea? * Cystoisospora canis infected? Crosstabulation

			Cystoisospora canis infected?		
			No	Yes	Total
Diarrhea?	Yes	Count	26	2	28
		Expected Count	27,0	1,0	28,0
	No	Count	57	1	58
		Expected Count	56,0	2,0	58,0
Total		Count	83	3	86
		Expected Count	83,0	3,0	86,0

Table 17. Statistical analysis of G. duodenalis *infected dogs with diarrhea x infection with* Cystoisospora canis

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,647 [a]	1	,199		
Continuity Correction [b]	,431	1	,512		
Likelihood Ratio	1,515	1	,218		
Fisher's Exact Test				,246	,246
N of Valid Cases	86				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is ,98.

b. Computed only for a 2x2 table

Hookworms

Table 18. Crosstabulation; G. duodenalis *infected dogs with diarrhea x infected with possible hookworms.*

Crosstab

			Hookworm infected?		
			No	Yes	Total
Diarrhea?	Yes	Count	12	16	28
		Expected Count	19,2	8,8	28,0
	No	Count	47	11	58
		Expected Count	39,8	18,2	58,0
Total		Count	59	27	86
		Expected Count	59,0	27,0	86,0

Table 19. Statistical analysis of G. duodenalis *infected dogs with diarrhea x infection with possible hookworms*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	12,779 [a]	1	,000		
Continuity Correction [b]	11,067	1	,001		
Likelihood Ratio	12,436	1	,000		
Fisher's Exact Test				,001	,001
N of Valid Cases	86				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 8,79.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	,385	,000
	Cramer's V	,385	,000
N of Valid Cases		86	

Capillaria

Table 20. Crosstabulation; G. duodenalis *infected dogs with diarrhea x infected with* Capillaria

Diarrhea? * Capillaria infected? Crosstabulation

			Capillaria infected?		
			No	Yes	Total
Diarrhea?	Yes	Count	27	1	28
		Expected Count	27,3	,7	28,0
	No	Count	57	1	58
		Expected Count	56,7	1,3	58,0
Total		Count	84	2	86
		Expected Count	84,0	2,0	86,0

Table 21. Statistical analysis of G. duodenalis *infected dogs with diarrhea x infection with* Capillaria

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,284 [a]	1	,594		
Continuity Correction [b]	,000	1	1,000		
Likelihood Ratio	,266	1	,606		
Fisher's Exact Test				,548	,548
N of Valid Cases	86				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is ,65.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	,057	,594
	Cramer's V	,057	,594
N of Valid Cases		86	

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