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The difference in estimated prevalences of *Ancylostoma tubaeforme* between Dutch indoor and outdoor household cats

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Abstract

In household cats infections with intestinal parasites are important because of both the cat's health and the public health as there can be a zoonotic risk. It is important to know the lifestyle of the cat, which parasites are present in the cat and which deworming regimen is applied by the cat owner. An infection with intestinal parasites may depend on these factors. One of the parasites that infects cats in the northern part of Europe, but which is perhaps less well known to veterinarians in the Netherlands, is the hookworm, *Ancylostoma tubaeforme*. In this study, 354 fecal samples from household cats in the Netherlands have been analyzed, using the CSF and Baermann technique for an estimation of the prevalence of *A. tubaeforme* and other intestinal parasites. Strongyle type eggs were cultured for determination of *A. tubaeforme* larvae. Because information about larval determination is lacking, the larval morphology is restricted to the genus *Ancylostoma* sp. and based on the larval morphology of *A. caninum*. Association between patent infections and the lifestyle of cats (indoor versus outdoor cats), infection with intestinal parasites, infection with *A. tubaeforme* and deworming (0-4 times a year versus > 4 times a year) were analyzed, using the Chi-square test.

183 participants have sent their cat's faeces to the laboratory of the Faculty of Vetrinary Medicine at Utrecht University. In total fecal samples of 354 individual cats were analyzed, of which 191 cats were indoor cats and 147 cats were outdoor cats, from 16 cats data was lacking. A total of 26 cats (7,3%) were found positive for intestinal parasites. 9 indoor cats (4,7%) were found positive for intestinal parasites and 15 outdoor cats (10,2%) were found positive for intestinal parasites, of which one cat was found positive for *A. tubaeforme* (0,3%). Outdoor cats have a significant higher prevalence of intestinal parasites than indoor cats. The questionnaire is answered by 177 participants for 338 individual cats. From the 338 cats, 325 cats were dewormed. From the 325 dewormed cats, 237 cats are dewormed at least once a year, 88 cats were dewormed without using a deworming schedule. Significantly more outdoor cats are dewormed more than four times a year than indoor cats. Furthermore, the association between deworming and intestinal parasites is statistical analyzed and dewormed cats have a significant higher prevalence of intestinal parasites than non-dewormed cats. However, 88 % of the participating cats are dewormed more than one month ago. The recent use of the anthelmintic and the most commenly used short-acting anthelmintic Milbemax could be an explanation for the significant difference.

For further research, more cats need to be sampled for more reliability of the conclusions. Research of the morphology of the larvae of *A. tubaeforme* is necessary for a complete determination and microscopic diagnosis with more certainty.

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1. Introduction

Cats are at the top of the list of the most commonly kept pet animals and are very popular in the Netherlands. Commissioned by the trade association of petfood manufacturers and petfood importers, the Dutch Petfood Industry (NVG) and trade association Dibevo, MarketResponse conducts research into the cat population in the Netherlands. A recent study reported that 2.6 million cats live in the Netherlands. In almost a quarter of the households lives one or more cats.[10] The majority of these households are families with children and live in or near the city, meaning that in one place several cats live close together. With the point of view to zoonotic risk and therefore the health for humans and animals as well, it is important to know the lifestyle of the cat, which parasites are present in the cat and which deworming regimen is applied by the cat owner. When cats become infected with parasites, it can also be a high risk for public health.

This research focuses on Dutch household cats. The lifestyle of the cat is examined for factors, such as environment. Research into the deworming schedule of the cats is performed, to examine the awareness of the risk for public health by an infection of intestinal parasites.

1.1 Lifestyle of the cat

An infection with intestinal parasites can affect the health of the cat and depends on various lifestyle factors. The environment is an important factor for infection with parasites. Cats living outdoors or living with other cats or dogs may be at greater risk of acquiring parasites.[2] The outdoor cat is free to cath a prey and defecate either inside or outside the house. An indoor cat lives in the house constantly and additionally has a reduced risk of acquiring parasites by eating a prey. The prevalence of intestinal parasites in Dutch cats has been researched by Koolwijk (2011). This study revealed that the amount of infected indoor cats was much lower than the amount of infected outdoor cats. Generally, outdoor cats have a higher risk of acquiring parasites.[11] The residence of the cats are also of importance. In rural environments, cats may capture more prey animals, however the city has a denser population. Asuming the close contact between cats in the city, cats can be at higher risk for infections. In addition to the environment and residence of the cat, meals given by the owner of the cat is also of importance concerning infections with parasites. Indoor cats has a reduced risk of acquiring parasites by eating a prey, however infections with intestinal parasites can occur by eating raw fish and raw meat. Furthermore, age and health status also play a role in infections with parasites. Aged cats, kittens and cats with reduced immunity are at greater risk than healthy adults.[2]

1.2 Deworming schedule

Cats can become infected with various intestinal parasites, which can be subdivided into two groups: helminths and protozoans. Some of the helminth can cause Viscerale Larva Migrans syndroom (VLM) and the Oculaire Larva Migrans syndroom (OLM) in humans. Deworming for cats is therefore not only important for the cat, but also for the public health.[11,18] The roundworm (*Toxocara* spp.) is the most important helminth and forms a zoonotic risk. The advice on anthelmintic treatment for cats in the Netherlands is based on this parasite. For the cats health, but also of minimizing public health risks, it is advised by the European Scientific Counsel Companion Animal Parasites (ESCCAP) to deworm a cat at least 4 times a year.[2] In a study by Janssen (2011), 54 participants were asked about the deworming schedule of the cat. The study revealed that the majority of cat owners do not have a deworming schedule for the cat. Furthermore, 14 participants dewormed their cat 2-3 times a year.

Most cat owners deworm their cat on veterinary advice, but it is unclear for what reason the cat is not dewormed or occasionally dewormed.[18] The ESCCAP has made no distinction in the advised deworming regimens between indoor or outdoor cats, while the ESCCAP also suggests that the infection thrives best where animals have access to outdoor environments.[2] By studying the difference in prevalence of intestinal parasites between indoor en outdoor cats, the deworming schedule might be customized for these groups. Furthermore, de advice on anthelmintic treatment is based on *Toxocara* spp., while other roundworms also may be of interest. One of these parasites is the less known hookworm in Dutch cats. The importance of this parasite can be taken in consideration to adjust the deworming schedule, after research on this parasite.

1.3 Hookworms

Hookworms are small nematodes belonging to the group of helminths and affect the small intestine of dogs and cats. The worms hooks up to the gut mucosa with their oral capsule and have a relevant blood-sucking activity, with anemia by iron deficiency as direct consequence. Worms may detach and move to new sites and reattach. Infected cats show deterioration in health and severe clinical signs. Knowledge of all hookworm infections is of importance because of the worldwide high risk for animal and human health. Hookworms are members of the family *Ancylostomatoidae* and the most important genus of this family is *AncylostomaI* sp..[3,12] An important intestinal parasite, maybe less known to Dutch veterinarians, is the hookworm *Ancylostoma tubaeforme*.

Four species of *Ancylostoma* are known in cats and dogs. These are the *Ancylostoma caninum*, *Ancylostoma braziliense*, *Ancylostoma tubaeforme* and *Ancylostoma ceylanicum*. The last three are found in cats, but only *Ancylostoma tubaeforme* is of importance in the northern part of Europe.[5] In a research by Mulder (2010), which took place in the surroundings of Breda in The Netherlands, the prevalence of cats infected with *Ancylostoma tubaeforme* was 6,4%.[4] A different genus of hookworms, which is less common in cats and more seen in dogs, is called *Uncinaria stenocephala.*

The most widespread of all hookworms in prevalence is *A. caninum* and is best known. This parasite is found in dogs, however it can also reside in the human gut, but here they do not develop any further. In a research by Traversa (2012) is reported that *A. caninum* can invade and parasitize humans or can penetrate the human skin, causing cutaneous larva migrans, however humans are not used as definitive host. Despite their importance in public health perspective, very little is known about precisely how these parasites interact with their hosts, especially about *A. tubaeforme*. Zoonotic cases of *A. tubaeforme* are not reported. In the research by Traversa (2012) there is no perspicuity about *A. tubaeforme* in people. The human skin is not penetrated, but little skin penetrations are possible. However, this is not seen as an effector of cutaneous larva migrans.[3,12] Since the zoonotic role of *A. tubaeforme* is unclear, this parasite is treated as a risk for public health. Hookworms found in cats and dogs in the 18th century, were described as *Strongylus tubaeforme* (cats) and *Sclerostoma caninum* (dogs). Later *S. caninum* was transferred to the genus *Ancylostoma*, but the hookworm of cats was overlooked by most investigators and the common hookworm of both cats and dogs was referred to *A. caninum* for nearly 100 years. In the 19th century it was found that the hookworm of the cat and the dog were species-specific, but were considered different strains of the same species. In the sequel of the research Biocca (Burrows, 1962) has found that there were two species rather than two strains of one species. A redescription of *A. tubaeforme* with morphological differences between the adults of these two species is made, however information

about larval determination is lacking.[13] Fecal samples is examined for eggs of *Ancylostoma* to diagnose hookworms. The prevalence in the research by Mulder (2010) is estimated by calculating the proportion of strongyle type eggs positive faecal samples over the total number of examined faecal samples.[4] However, the morphological determination of *A. tubaeforme* eggs is very difficult because of the similarity between *A. tubaeforme* eggs and strongyle type eggs origing from the consumption of prey animals (passage). In several studies, a morphological differentation on eggs of *Ancylostoma* species carried out through polymerase chain reaction (PCR).[20] On behalf of the research into the prevalence of *A. tubaeforme* in Dutch household cats, culturing is necessary to identify the larvae of *A. tubaeforme*. Considering the sparse knowledge of *A. tubaeforme*, the larval morphology must be derived from *A. caninum* in order to make comparison to the genus *Ancylostoma*. The fact *A. tubaeforme* was seen for years as the same species as *A. caninum*, larvae of *A.caninum* are the perfect match for comparison. These species are almost identical.[5]

1.4 Life-cycle of *Ancylostoma tubaeforme*

Hookworms have a direct life-cycle. *A. tubaeforme* is broadly similar to the known hookworm in dogs (*A. caninum*) and information about the life-cylce can be derived from this hookworm. Transmammary transmission has not been demonstrated for *A. tubaeforme*.[5,12] Adult hookworms live in the small intestine of the cat and feed on blood in the mucosa. Heavy infections may lead to anaemia, a poor coat and reduced growth.[2]. Pathogenicity of *A. tubaeforme* is considerable, because of the probably low infections in the Netherlands. However, as mentioned earlier, diagnosis of *A. tubaeforme* is difficult and in a lot of cases, treatment will be based on symptoms and analyzing the faeces on worm eggs, wihtout carrying out further research which species.
In figure 1, the life-cycle is shown, followed by a description of various stages in the cycle of *A. tubaeforme*.

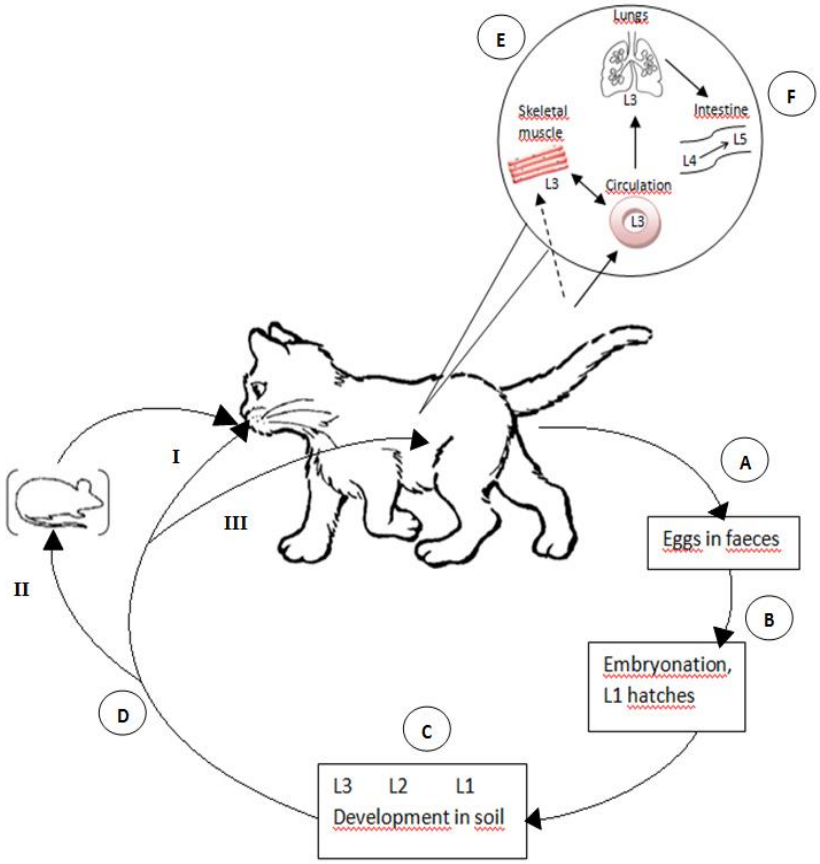

Figure 1: Life-cycle of *Ancylostoma tubaeforme*

A. Eggs are excreted in host faeces. [3]
B. The eggs develop in the faeces to first stage larvae (L1) in the environment under the influence of moisture and heat. [3]
C. The hatched first-stage larvae (L1) feed on micro-organisms in the soil and develop into L2 larvae and finally they become infective as a non-feeding third stage larvae (L3). In this stage, the larvae are enveloped in the detached outer cuticular sheath, which is left over from the second molt.[3,12]
D. The cat can be infected by ingestion of the L3, direct (**I**) or in paratenic hosts, such as rodents (**II**). In both cats and paratenic hosts, the L3 larvae can develop directly in the fourth stage larvae (L4) in the gut, whereas others penetrate the mucosa to undergo tracheal or somatic migration. However, the relative importance of the direct development, it is unlikely to be a significant route of infection. Another route of infection is through skin penetration (**III**), through hair follicles and through sebaceous glands and apocrine sweat glands. Eventually the larval stages enter the blood or lymphatic capillaries.[3,12,14]
E. Some skin-penetrating L3 larvae possibly migrate directly into underlying skeletal muscle. The L3 larvae which end up in the circulation through penetration, recommences feeding on exposure to plasma and the worms resume growth. From the circulation L3 larvae can take two migration routes, as mentioned earlier.

    Somatic migration: The larvae disperge through tissues and become hypobiotic in skeletal muscle fibers. The hypobiotic L3 reactivate prior to the onset of the warm, wet season and enter the gut when conditions are optimal for transmission to develop into adult worms. [3]

Tracheal migration: The larvae pentrate alveoli to get into the lungs. From there the larvae migrate through the airway and are coughed up and swallowed. The larvae eventually enters the intestines.[3]

F. The L3 will develop directly to a fourth stage (L4) in the gut. The adult hookworm (L5) feeds by sucking clumps of villi and may live for 4 to 24 months in the small intestine of the cat. The female adult worms produce eggs.[3,7]

Immunity against *A. tubaeforme* develops after exposure, but is unlikely to be absolute. Cats with small numbers of worms will not show any symptoms but they will contaminate their environment unnoticed. The pre-patent period lasts 2-3 weeks, but the patent period can be prolonged for 7 months to 2 years, depending on the immune status of the cat.[2,7]

1.5 Aims and hypotheses

The purpose of this study is to provide an estimation of the prevalence of *A. tubaeforme* in Dutch household cats. Beside the estimation of *A. tubaeforme*, the prevalence of intestinal parasitic infections will be appointed. As outdoor cats might show a significant higher prevalence of intestinal parasitic infections than indoor cats, it will be examined whether a difference in prevalence of *A. tubaeforme* exists between indoor and outdoor cats.

The association between deworming and infection with intestinal parasites will also be appointed. Furthermore, a distinction in the frequency of deworming is made and the association between the frequency and lifestyle of the cat will be examined. The cat owners will be asked to fill in a questionnaire to collect information about the lifestyle and deworming schedule of the cat.

Three aims of the study are formulated.

1. Estimating the pevalence of *Ancylostoma tubaeforme* in Dutch houshold cats.
2a. Studying the association between the lifestyle of cats (indoor versus outdoor cats) and infection with intestinal parasites.
b. Studying the association between the lifestyle of cats (indoor versus outdoor cats) and infection with *Ancylostoma tubaeforme*
3a. Studying the association between deworming and infection with intestinal parasites.
3b. Studying the association between deworming (0-4 times a year versus > 4 times a year) and lifestyle of the cats (indoor versus outdoor cats)

On the basis of the formulated aims, hypotheses for the second and third aim are formulated.

2a) H_0: There is no association between the prevalence of intestinal parasites and the lifestyle of the cat (indoor versus outdoor cats)

2b) H_0: There is no association between the prevalence of *Ancylostoma tubaeforme* and the lifestyle of the cat (indoor versus outdoor cats)

3a) H_0: There is no association between deworming and infection with intestinal parasites.

3b) H_0: There is no association between deworming (0-4 times a year versus > 4 times a year) and lifestyle of the cats (indoor versus outdoor cats)

2. Material and Methods

2.1 Population and sample size

The sample size is based on the knowledge by literature. In a research of Mulder (2010) about cats from shelters in and around Breda, a prevalence of *A. tubaeforme* of 6,4% is found.[4] Another research in Germany of Barutzki and Schaper (2011) about owned cats reported a prevalence of 0,2% in 8.560 cats. This research shows that the prevalence of owned cats is lower than stray cats or cats from shelters, however the prevalence is derivative from the cat population of Germany.[1] In a research of Overgaauw (1997) the prevalence of *A. tubaeforme* has not been studied, however a significant association between stray cats and houshold cats is reported.[15]

In this study a distinction between indoor and outdoor household cats in The Netherlands is made. Information from the cat owners about the deworming schedule and lifestyle of the cat is needed. Stray cats do not have an owner and both stray cats and cats from shelters do not live in a household. Therefore, stray cats and cats from shelters are not discussed in this study. Considering the prevalences reported in the previous studies, mentioned earlier, a prevalence between 1% and 6% in this study is expected. The sample size is calculated with a webbased epidimiological tool from Ausvet animal health services (table 1).[6] Assuming a prevalence of 4% with a confidence interval of 95% and a precision of 5%, 150 cats are needed.

Inputs

Assumed true prevalence	0.04
Sensitivity	0.95
Specificity	0.95
Population size	Large population
Confidence	0.95
Desired precision	0.05

Results

Sample size required

	Sample size
Large population	150

Table 1: Sample size to estimate true prevalence

To ensure that there are no large groups with similar lifestyles and environment, each participant can participate with a maximum of 5 cats. Cats from the same household are clustered, therefore it would be ideal to have 150 participants.

2.2 Collecting faeces and information

The number of cats needed for research are obtained by contacting cat owners directly or by contacting unknown cat owners through social media (Appendix I). Furthermore, affiches are placed in public places, such as petshops and animal clinics (Appendix II). Anyone who resides in the Netherlands can participate in the study. After registration, the participants were contacted via email and the address and participating cats are registered in an Excel file. Every owner participating in the study received an individual number for correspondence during the study. A package was sent to the

address of the participant to collect the fecal samples and send it to the Faculty of Veterinary Medicine. Instructions on how to collect and send the faeces properly is includes in the package. (Appendix III). Only one sample collected from each cat is needed to diagnose eggs of *A. tubaeforme*. If the participant was not in the opportunity to send the package directly, the participant is advised to keep the collected faeces for a maximum of one day cool. In order to reduce the workload, the participants are divided into groups with an assigned deadline to spread the income of the fecal samples over a number of weeks.

2.2.1 Questionnaire

To receive information about the lifestyle of the cat, the used dewormer and the deworming schedule, the owners are asked to complete a questionnaire (Appendix IV). The questionnaire is web based and the outcome of the corposcopical analysis was sent after the questionnaire has been completed.

Based on the information, given by the owner, cats are classified as either an 'indoor cat' or an 'outdoor cat'. A comparison in prevalence between these two groups is made.
If it has been indicated in the questionnaire that the cat does not come outside, the cat is classified as an indoor cat. If it has been indicated in the questionnaire that the cat does come outside, the cat is classified as an outdoor cat, with the exception on cats only resides on a balcony where no other cats are able to access and the cats has a reduced risk of acquiring parasites by eating a prey. These cats are aslo classified as an indoor cat, because of the equally chance of infection with intestinal parasites as within the case of a cat who never comes out the house.

2.3 Corposcopical analysis

When the fecal samples in the lab were received, the faeces were examined the same day. All faeces were examined using the Centrifuge-Sedimentation-Flotation technique (CSF) and the Baermann technique. Furthermore, the sample is cultured when the Baermann was positive for strongylus type eggs. A description of these techniques is given in the paragraphs below. All samples were kept for at least two days in the refrigerator. The samples were discarded if a negative result came out from the research.

2.3.1 Centrifuge-sedimentation-flotation technique

The CSF technique is an active flotation method for semi-quantitative faecal examination and can be done on worm eggs, cysts and oocysts. This method makes use of the difference in specific gravity (s.g.) between worm eggs, oocysts, cysts and other fractions of the sample.[16] A protocol has been drawn up for carrying out this technique (Appendix V). The method is carried out in two parts. In the first part of the protocol, a suspension is of the faecal sample is obtained. Four grams of faeces is mixed with 55 ml tap water in a mortar and mashed up to a liquid substance being filtered and large parts remain behind in the sieve. The suspension is poured into a test-tube, which is placed into a centrifuge, and centrifuged for two minutes at 3000 rpm. All particles with a s.g. bigger than 1 (inclusive the worm eggs, oocysten and cysten) precipitate, and the water with the lighter particles is called the supernatant and is poured off. In the second part of the protocol, the sediment is suspended in an extraction liquid. In this study of a sucrose solution having a density of 1.28 g / cm3 is used. The test-tube is placed back into the centrifuge and is refilled with the sucrose-solution until

a slight round meniscus appears. On the top of the round meniscus, a coverslip is placed and the test-tube is again centrifuged for two minutes at 3000 rpm. Because the worm eggs, oocysten and cyts have a lower density than the liquid, these abut against the coverslip. When the coverslip vertically is removed after centrifuging, the coverslip contains the worm eggs, protozoan oocysten and cycts and is placed on an object glass for analysis under a ligth-microscope. The object glass was systematically viewed with a magnification of 100-400x. In case of two of more same worm eggs, protozoan oocysten or cycts are found, the cat is regarded as positive.

In general, a test-tube containing one sample. When there are more than 16 samples on the same day received two samples were put in one test tube, provided that the sample contains sufficient faeces to be able to repeat the method if the result is positive. In this way, all the samples on the day of receiving could be examined. In each test-tube, a ratio of 3 grams per sample with 82 ml of tap water is used. In case the examination leads to a positive result, the two samples were each separately analyzed to determine which cat is positive.

2.3.2 Baermann technique

The Baermann technique is used to diagnose lung worms. With the Baermann technique the moving larvae are separated from non-moving fecal components.[4,16] A protocol has been drawn up for carrying out this technique (Appendix VI). Settlings glass are used, which terminate in a point and have a fine-mesh filter wit a pasage diameter of 45 µm (figure 2). The Baermann glass is filled with tap water untill half of the filter is under water. Three gram faeces is placed onto the filter, whereupon the Baermann should remain at room temperature for 24 hours. Within this time the larvae crawl out of the faeces down through the filter. After 24 hours, the fluid in the point of the glass is transported to a small embryo cube by using a pasteur pipette. The fluid is examined with a stereo-microscope for the presence of larvae at a magnification of 32-40x.

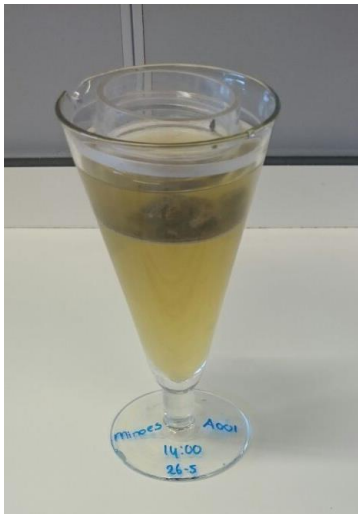

Figure 2: Baermann glass

2.3.3 Culturing larvae

Once strongylus type eggs are found by using the CSF technique, the sample was cultured to identify the larvae of *A. tubaeforme*. No guideline is known for the amount faeces to be used for culturing larvae by cats. As the feces of cats consisted of small amounts, the remainder of the sample is used. The faeces is put into a glass jar with sawdust. The sample was kept moist by the addition of tap water. The glass jar was covered with a lid that was not tightened, so that oxygen could reach inside the glass jar. The sample was for nine days at room temperature and the humidity is checked every two days. After nine days, the glass jar is completely filled with tap water and with a petri dish on top it is entirety put upside down (figure 3). The petri dish is filled up with tap water and after one day the water from the petri dish is filled into a falcon-tube by using a pasteur pipette. After one hour, the fluid on the bottom of the falcon-tube is put into a embryo cube and is


Figure 3: Glass jar with petri dish

analyzed under a stereo-microscope. When larvae are found, a few larvae were dropped on a microscope slide by using a a pipette. Iodine is used for killing and color of the larvae. After a cover glass is placed on top of the microscope slide, the larvae were analyzed under a light-microscope.

2.4 Determination key of *Ancylostoma tubaeforme* larvae

The determination of *A. tubaeforme* is conducted in two steps. Eggs of *Ancylostoma* sp. are similar to other strongyle type worm eggs. In the first step, all strongyle type worm eggs are measured and compared with the reference sizes of *Ancylostoma* sp. eggs.[5] Strongyle type eggs are long, oval and approximately 40-85 µm.[19] Eggs of *Ancylostoma* sp. are microscopic similar, but the size can be different. The reported ranges of size of *A. tubaeforme* eggs are 56-75 x 34-47 µm, this is similar to *A. caninum*, but smaller than other *Ancylostoma* sp.[5] In the second step, strongyle type eggs, fitting the size of *A. tubaeforme,* are put on culture to distinguish harvested third stage larvae between *Ancylostoma* sp. and other genera. Furthermore, a determination of *A. tubaeforme* has to be made. However, as mentioned earlier in the introduction, little is known about the morphology of the infective third stage larvae of *A. tubaeforme*. Therefore, the larval morphology is restricted to the genus *Ancylostoma*.

To identify the harvested third stage larvae as a genus of *Ancylostoma*, the morphology of the infective third stage of *A. caninum* is used and is described in a study by Nichols (1955). The length and width of *A. caninum* is respectively 660 x 22 µm, with a maximum diamater of 24 µm. The mean dimensions of the larvae are described in table 2. The cuticle forms the outer layer of the larvae and has only faint circulair annulations. The alae are protuding ridges and are formed double longitudinal. The oral apparatus consists of a buccal capsule and functions in the initial uptake of food into the alimentary tract. Heavy cuticular walls line the capsule and are directly continous with the cuticular lining of the esophagus. Dorsal of the esophagus, glands extend as a column of granules the entire length of the esophagus from the bulb to the buccal capsule. The body cavity is filled with ganglionic nuclei which are especially numerous posterior tot the nerve ring. A Drawing of the infective third stage larvae of *A. caninum* is shown in Appendix VII.[17]

Table 2: Mean dimensions in microns of infective third stage larvae of *Ancylostoma caninum*

Length		**Width***		**Esophagus**	**Ex. pore**	**Anus**
Average	Range	Average	Range	Average	Average	Average
664	576-967	22	20-24	170	124	76

* Width, measured at esophago-intestinal junctions; Esophagus, length from mouth to esophago-intestinal junctions; Ex. pore, distance from mouth to excretory pore; Anus, distance from anus to tip of tail

Nichols (1995) made a determination table based upon four characteristics: the relative size of the larva; type of intestine; the presence and type or lack of lateral alae; the presence and size or lack of excretory columns. The determination table is illustrated in table 3.[17]

Table 3: Determination table

	Description	Furthermore	Species
1	Diameter at mid-gut 14-16 µm Lateral alae prominent	2	
	Diameter at mid-gut greater than 26 µm Lateral alae present or absent	5	
	Diameter at mid-gut 14-17 µm Lateral alae minute and single	-	Early second stage *Ascaris lumbricoides*
2	Lateral alae single	3	
	Lateral alae double	6	
3	Single gut cell; posterior excretory columns large, wel defined Minute central excretory canaculi frequently present	4	
	Intestine with prominent lumen and composed of 2 or 3 cells Excretory columns small, well difined	-	Advanced second stage *Ascaris lumbricoides*
4	Diameter at mid-gut 16 µm or less	-	Early second stage *Toxocara cati*
	Diameter at mid-gut 18-22 µm	-	Early second stage *Toxocara canis*
5	Diameter at mid-gut 26-50 µm Lateral alae absent; excretory columns reduced or absent	-	Advanced third stage *Necator americanus*
	Diameter at mid-gut 26-50 µm Lateral alae prominent; excretory columns wit cross-section are equal to or greater than intestine	-	Second to third stage *Ascaris lumbricoides*
6	Diameter at mid-gut 22-24 µm; excretory columns large, Well defined; central excretory canaliculi lacking	-	Early third stage *Ancylostoma caninum*
	Diameter at mid-gut 14-26 µm Excretory columns reduced or lacking	7	
7	Diameter at mid-gut 22-26 µm 2 gut cells filling the body cavity	-	Early third stage *Necator americanus*
	Diameter at mid-gut 14-16 µm 2 gut cells not filling the body cavity	-	Early third stage *Strongyloides stercoralis*

Based on the determination table and the characteristics of *A. caninum*, a comparison with larvae of *Ancylostoma* sp. is made in this study when the infective third stage larvae contains double lateral alae, has a width of 22-24 µm diameter at the mid-gut, and the circulair annulation are present.

2.5 Statistisc analysis

2.5.1. Confidence interval

The prevalence of *A. tubaeforme* and other parasites are determined in this study. A statistical analysis is needed to find out whether the results are reliable. The number of positive cats must be divided by the total number of sampled cats (n) to determine the fraction of positive cats (p) within the sample survey, which is called the prevalence (π). In order to determine the reliability of this result a calculation with a confidence interval is needed. Assuming the sample survey is representative for the population of household cats in the Netherlands, a 95% confidence interval of the prevalence is calculated according to the subjoined formula: [8,9]

$$p - 1{,}96\sqrt{((p(1-p))/n)} < \pi < p + 1{,}96\sqrt{((p(1-p))/n)}$$

2.5.2. Association between two variables

Beside the prevalence of intestinal parasites in Dutch household cats, it will be examined whether the prevalence of intestinal parasites is higher in outdoor cats than in indoor cats. The variables lifestyle, deworming and nutrition may also are of importance in the prevalence of intestinal parasites. Cross tabs were made in SPSS 16 for Windows to find out whether there is an association between these variables and an infection with intestinal parasites. The Chi-square test is used to examine the statistical significant association between two variables. Applying the Chi-square test is partly dependent on the height of the expected frequencies and may be used only if it measure up to the following two conditions: all expected frequencies have to be larger or equal to 1 and a maximum of 20% of the expected cell frequencies can be smaller than 5. This means that all expected values must be above 5, in a cross table of 2x2.[8,9]

3. Results

3.1 Prevalence of intestinal parasites

In this study 183 participants have sent their cat faeces. In total 354 individual fecal samples were analyzed. 26 cats (7,3%) were found positive for intestinal parasites, of which 1 cat was found positive for *A. tubaeforme* (0,3%). Eight cats were found positive for multiple intestinal parasites (2,3%). A map displaying the distribution of the participants can be found in appendix VIII.

From a total of 354 individual participating cats, seven were found positive for *Giardia* (2,0%), seven for *Isospora* (2,0%), six for *Toxocara cati* (1,7%), three for *Sarcocystis* (0,8%), three for *Capillaria* (0,8%), one for *Cystoisospora* (0,3%), one for *Toxoplasma gondii* (0,3%), one for *Opisthorchis* (0,3%) and one for *Toxascaris leonia* (0,3%) by using the CSF technique. Two cats were found positive for *Aelurostrongylus* (0,6%) and one cat, named Nell, was found positive for an unknown parasite (0,3%) by using the Baermann technique. The faeces of Nell was examined twice. The first time, one larvae was found by using the Baermann technique. However, this larvae died during the transport from the small embryo cube to a microscope slide for further determination. The larvae was damaged and a second sample of the faeces was needed. Unfortunately, no larvae were found the second time by using the Baermann technique. The larvae found the first time was unkown, therefore Nell should not be found negative for intestinal parasites. An overview of the number of positive samples, the percentgae of positive samples and the 95% confidence interval of intestinal parasites which are found in this study, is shown in table 4.

Table 4: Intestinal parasites found during this study

Parasite	Number of positive samples	% of positive samples	95% confidence interval
Intestinal parasites in total	26	7,3 %	4,6 % - 10,0 %
Giardia	7	2,0 %	0,5 % - 3,5 %
Isospora	7	2,0 %	0,5 % - 3,5 %
Toxocara cati	6	1,7 %	0,4 % - 3,0 %
Sarcocystis	3	0,8 %	0 % - 1,7 %
Capillaria	3	0,8 %	0 % - 1,7 %
Aelurostrongylus	2	0,6 %	0 % - 1,4 %
Cystoisospora	1	0,3%	0 % - 0,9 %
Toxoplasma gondii	1	0,3%	0 % - 0,9 %
Opisthorchis	1	0,3%	0 % - 0,9 %
Toxascaris leonia	1	0,3%	0 % - 0,9 %
Ancylostoma tubaeforme	1	0,3%	0 % - 0,9 %
Unknown	1	0,3%	0 % - 0,9 %

3.1.1 Prevalence indoor versus outdoor cats

Out of the 354 individual participating cats, 191 cats were classified as indoor cats (54%) and 147 cats were classified as outdoor cats (41,5%). Seven participants have partly or totally not completed the questionnaire, therefore 16 cats were classified as unknown (4,5%). Nine of the positive cats were classified as indoor cats (4,7%) and 15 of the positive cats were classified as outdoor cats (10,2%). From the unknown group, two cats were positive (28,6%).

Table 5 illustrates which intestinal parasites were found within the group of outdoor cats, indoor cats and the unknown group. Furthermore, the number of positive samples, the percentage of positive samples and the 95% confidence interval is shown.

Table 5: Intestinal parasites found in indoor cats and outdoor cats

Parasite	Number of positive samples	% of positive samples	95% confidence interval
Indoor cats (191)			
Intestinal parasites in total	9	4,7 %	1,7 % - 7,7 %
Giardia	4	2,1 %	0,1 % - 4,1 %
Isospora	3	1,6 %	0 % - 3,4 %
Toxocara cati	1	0,5 %	0 % - 1,5 %
Sarcocystis	2	1,0 %	0 % - 2,4 %
Aelurostrongylus	1	0,5 %	0 % - 1,5 %
Outdoor cats (147)			
Intestinal parasites in total	15	10,2%	5,3 % - 15,1 %
Giardia	1	0,7 %	0 % - 2,0 %
Isospora	4	2,7 %	0,1 % - 5,3 %
Toxocara cati	5	3,4 %	0,5 % - 6,3 %
Sarcocystis	1	0,7 %	0 % - 2,0 %
Capillaria	3	2,0 %	0 % - 4,3 %
Aelurostrongylus	1	0,7 %	0 % - 2,0 %
Cystoisospora	1	0,7 %	0 % - 2,0 %
Toxoplasma gondii	1	0,7 %	0 % - 2,0 %
Opisthorchis	1	0,7 %	0 % - 2,0 %
Toxascaris leonia	1	0,7 %	0 % - 2,0 %
Ancylostoma tubaeforme	1	0,7 %	0 % - 2,0 %
Unknown Group			
Intestinal parasites in total	2	28,6 %	6,5 % - 50,7 %
Giardia	2	28,6 %	6,5 % - 50,7 %

3.1.2 The association between intestinal parasites and lifestyle

The prevalence of intestinal parasites is compared between indoor and outdoor cats, by using the Chi-square test. An associaton between a positive outcome on intestinal parasites and the lifestyle of the cat has been examined. The nul-hypothesis is formulated as: There is no association between the prevalence of intestinal parasites and the lifestyle of the cat (indoor versus outdoor cats). The test results of the Chi-square test is displayed in appendix IX A. According to the significance, with a p-value < 0,05 found in the Chi-square test, the nul-hypothesis must be rejected. This implies the association between the prevalence of intestinal parasites and the lifestyle of the cat. Outdoor cats have significantly a higher prevalence of intestinal parasites than indoor cats.

Only one cat has been found positive for *A. tubaeforme*, therefore no association can be made between the prevalence of *A. tubaeforme* and the lifestyle of the cats. The second hypothese given in chapter Material and Methods as 2b become due.

3.1.3 Larvae culture

From 26 cats which were found positive for intestinal parasites, one cat had stongyle type eggs (figure 4). The eggs were measured 62-65 µm and fitting the size of *A. tubaeforme* (56-75 x 34-47 µm). No other strongyle type eggs were found in this study.

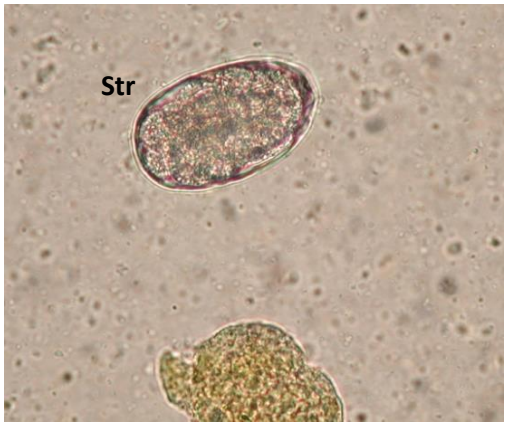

Figure 4: Strongyle type egg (Str)

The sample is cultured and after nine days, two larvae were fixed for determination. The first larve had a length of 650 µm. A picture of the first harvested larve is given in figure 5. The diameter at the mid-gut is 24 µm and double lateral alae are prominent. The second larve had a lenght of 640 µm. The diameter at the mid-gut is 20 µm and double lateral alae are prominent. Drawings of the harvested, fixed larvae, are illustrated in appendix X. The simulacrum of both larvae was too obscure to make a good distinction in the body cavity. Determination based on the large, wel defined, excretory columns and central excretory canaliculi lacking, was impossible. However, slight faint circulair annulations of the cuticle were vissible. Based on the morphology and determination table of *A. caninum*, there are no reasons for non-classifying the harvested larvae as *Ancylostoma* sp.

Figure 5: First harvested larve

3.2 Questionnaire outcome

Based on the questionnaire, answered by the 177 participants for 338 individual cats, the lifestyle, and deworming of the cats are statistic analyzed. The 16 cats of which no information is known, are not further considered.

3.2.1 Age of the cats

The cats are divided into six groups for registering the age of the cats: younger than six months, six months up to one year, two year up to five years, six years up to nine years, ten years up to thirteen years, and fourteen years and older. From the 338 individual cats, 132 cats (39%) are two year up to five years, and is the biggest group of age. Five cats (1%) are younger than six months and 21 cats (6%) is fourteen years or older. The distribution by age is illustrated in appendix XI A.

3.2.2 Deworming

From the 177 participants, who answered the questionnaire, 169 partipants deworm their cat(s). Seven participants do not deworm their cat(s). A notable fact is that one participant deworm one of her cats, while the other cat is not dewormed. In total 325 cats are dewormed and 13 cats are not dewormed. An overview of deworming the cat is illustrated in table 6. The most commonly used anthelmintic is Milbemax. 144 participants (85,2%) give their cat an anthelmintic treatment with Milbemax.

Table 6: Deworming of the cat

Participants: 177*	169,5	7,5
	Dewormed	**Not dewormed**
Total cats: 338	325	13

* One participant with more than one cat in the household is split up. One cat is dewormed, the other cat is not dewormed.

From the 325 dewormed cats, 88 cats were dewormed without using a deworming schedule, 237 cats were dewormed by using a deworming schedule. These cats are categorized in: one time a year, two to three times a year, four times a year and more than four times a year. From 325 cats, 40 cats were dewormed one time a year, 112 cats were dewormed two to three times a year, 77 cats were dewormed four times a year, eight cats were dewormed more than four times a year. From the last group, four cats were dewormed 12 times a year and one cat is dewormed 6 times a year. One cat is dewormed 4 times a year, but is dewormed extra in case of eating a prey animal. Another cat is only dewormed, based on the faeces of the cat. One participant is in the possession of a kitten and deworm the cat temporarily 2 times a week.

To determine the last time the 325 cats had been dewormed, eight categories were made: less than one week, 1-2 weeks, 2 weeks upto 1 month, 1-2 months, 2-3 months, 3-4 months, 5-6 months, more than 6 months ago. From the 325 cats, 4 cats were dewormed less than one week, 10 cats 1-2 weeks ago, 25 cats 2 weeks upto 1 month, 61 cats 1-2 months, 60 cats 2-3 months, 47 cats 3-4 months, 30 cats 5-6 months, 85 cats more than 6 months ago and three cats were classified as unknown. An illustration of the last time of deworming can be found in appendix XI B.

From the 338 participating cats, most of the cats were dewormed. To study the association between deworming and infection with intestinal parasites, statistic analysis is conducted.

The association between deworming and intestinal parasites

The nul-hypothesis is formulated as: There is no association between deworming and infection with intestinal parasites. The test results of the Chi-square test is displayed in appendix IX B. Acording to the significance, with a p-value < 0,05 found in the Chi-square test, the nul-hypothesis must be rejected. This implies the association between the prevalence of intestinal parasites and deworming the cat. Dewormed cats have significantly a higher prevalence of intestinal parasites than non-dewormed cats.

Several reasons are given by the eight participants, who do not deworm their cats. Table 7 and figure 6 give an illustration of positive cats between indoor and outdoor cats which are dewormed, respectively not dewormed. Four participants do not deworm their cat, because the cat do not come outside the house. From these four participants, one participant was also not thinking about the use of deworming and one participant expresses to give no unnecessary chemical treatments. One participant do not deworm their cat, because the cat shows no symptoms. One participant indicates that deworming takes to much effort. One participant indicates that deworming is not necessary and one particpant has an unknown reason for not deworm their cat.

Table 7: Crosstable of positive cats between indoor and outdoor cats

	Dewormed	**Not dewormed**
Indoor cat	183	8
Positive	10	0
Outdoor cat	142	5
Positive	13	1

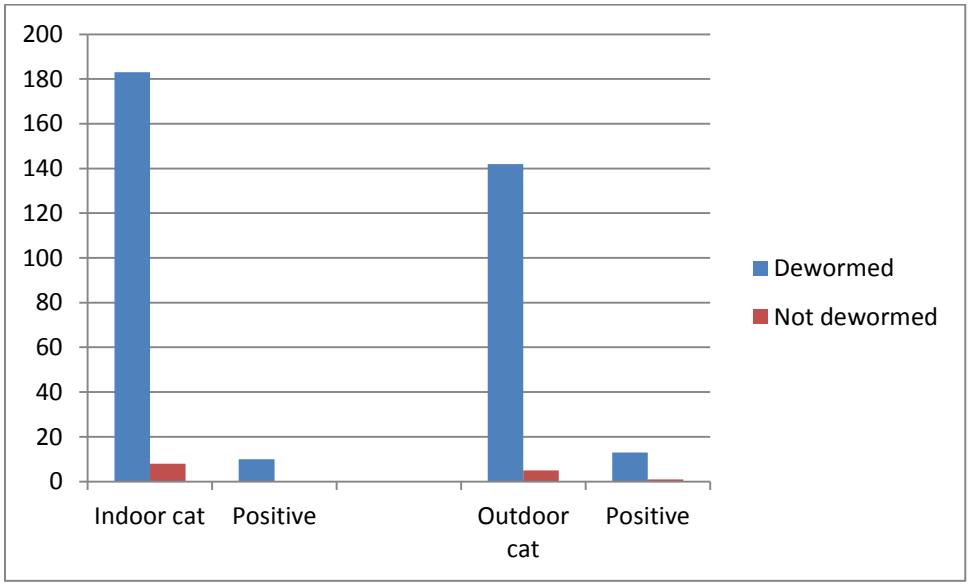

Figure 6: Diagram of positive cats between indoor and outdoor cats

As mentioned aerlier, the participants were asked about the deworming schedule that is used for their cats. The largest group is dewormed 2-3 times a year, for both indoor and outdoor cats. An illustration of the deworming schedule compared with indoor and outdoor cats is displayed in appendix XI C. The ESCCAP advises to deworm a cat at least 4 times a year. To study the association between deworming (0-4 times a year versus > 4 times a year) and lifestyle of the cats (indoor versus outdoor cats), statistic analysis is conducted.

The association between deworming and lifestyle

The nul-hypothesis is formulated as: There is no association between deworming (0-4 times a year versus > 4 times a year) and lifestyle of the cats (indoor versus outdoor cats). The test results of the Chi-square test is displayed in appendix IX C. Acording to the significance, with a p-value < 0,05 found in the Chi-square test, the nul-hypothesis must be rejected. This implies the association between the deworming schedule and lifestyle the cat. Outdoor cats are dewormed more than four times a year than indoor cats.

Discussion

The prevalence of intestinal parasites of 7,3 % found in this study is low, compared with the overall prevalence's reported earlier in the Netherlands. In a research of Mulder (2010) a prevalence of 26,6 % was found. In other Dutch studies by Koolwijk (2011) and Janssen (2012) a prevalence of intestinal parasites of 21,5 %, respectively 18,5 % was found. In a study of Overgauw, et al. (2009) a prevalence of 22,7 % was found. The difference between the estimated prevalence in this study and the reported prevalences can be explained by a possible difference in the population of the studied cats. In this study the samples are derived from Dutch household cats. All faecal samples in the research of Mulder (2010) are derived from cats living in a shelter. In a study of Overgaauw (1997), a significant difference of the prevalence of intestinal parasites between stray cats and houshold cats is reported. Stray cats have a significant higher prevalence of intestinal parasites than household cats.[15] However, all faecal samples in the researches of Koolwijk (2011), Janssen (2012) and Overgaauw, et al. (2009) are derived from household cats and the reported prevalences of intestinal parasites is higher than the prevalence found in this study. Therefore, the difference in the population of the studied cats cannot be an explanation of the low prevalence of intestinal parasites.

The low prevalence in this study can relying on other multiple factors. One of these factors is the deworming schedule of the cat and the period between testing and the previous anthelmintic treatment of the cats. However, the recent use of anthelmintics cannot be an explanation of the low prevalence of intestinal parasites, because 88 % of the participating cats are dewormed more than one month ago. The most commonly used anthelmintic is Milbemax which has an activity that lasts 2-3 days. Parasites with a prepatent period of 2-3 weeks (such as *A. tubaeforme*) should be detectable after one month of deworming. While looking to the association between deworming and the prevalence of intestinal parasites, another fact can be noticed. From the 13 non-dewormed cats, 1 cat was found positive for intestinal parasites. From the 325 dewormed cats, 23 cats were found positive for intestinal parasites. The association is satistical analyzed, dewormed cats have a significant higher prevalence of intestinal parasites than non-dewormed cats. This is contradictory in contrast to the guideline from the ESCCAP, where it is stated that the infection with parasites can be combated by deworming.[2] However, the used anthelmintic, the activity of the anthelmintic and the recent use of the anthelmintic is of importance for the prevalence on intestinal parasites. The commonly used anthelmintic and the recent use of the anthelmintic in this study, which was mentioned earlier, could be an explanation for this contradiction.

Another possible factor that could be causing this low prevalence is the used method for coproscopical examination. The methodology that is used in this study, is the same as in the previous mentioned studies with a higher prevalence of intestinal parasites. Therefore the methodology can also not be an explanation of the low prevalence found in this study. Another theory for the low prevalence of intestinal parasites, is a possible seasonal effect of shedding of stages of intestinal parasites. Temperature and moisture are important factors for development of parasites and are optimal in springtime.[23] Cats are seasonally poly oestrous and when giving birth the immune status is reduced.[22] However, information about the seasonal effect of cats shedding parasite eggs or (oo)cysts is lacking.

Besides the low prevalence of intestinal parasites, an accompanying low prevalence of *A. tubaeforme* is found during this study. One cat (0,3%) was found positive for *A. tubaeforme*. A study in Germany of Barutzki and Schaper (2011) reported a prevalence of *A. tubaeforme* of 0,2% and ratify the low prevalence found in this study. In a research by Mulder (2010), which took place in the surroundings of Breda in The Netherlands, the prevalence of cats shedding eggs of *Ancylostoma tubaeforme* was 6,4%.[4] However, only the CSF-technique and Baermann in both studies are used for diagnosis, assuming that strongyle type eggs are derived from *A. tubaeforme*. A distinction should have been made between *A. tubaeforme* eggs and strongyle type eggs origing from the consumption of the intestines of prey animals (passage). This distinction can be made by culturing the eggs for determination. In the study of Mulder (2010) and Barutzki and Schaper (2011) it is not proven that the strongyle type eggs actually are derived from worms of *A. tubaeforme*. In this study a population size for sampling was calculated based on the *Ancylostoma* prevalence of 6,4% which was found in the Ducth study of Mulder (2010). Probably, the true prevalence in the study of Mulder (2010) is lower.

Literature about *Ancylostoma* sp. can be found worldwide. However, knowledge about the species *Ancylostoma tubaeforme* is scarce.[13] The lack of knowledge about the morphology of the larvae of *A. tubaeforme* made it difficult to carry out a properly determination of the harvested larvae. Most studies use a PCR-test (polymerase chain reaction) for a specific identification of the eggs or larvae. The PCR is a sensitive and reliable method, but also expensive and is not commonly used as a first option in veterinary clinics. Knowledge of precise determination based on larval morphology is needed. A redescription of the morphology of adult stages of *A. tubaeforme* is made, however information about larval determination is lacking.[13] Therefore a conclusive determination of *A. tubaeforme* is not possible for our research setting. In this study the positive sample is cultured to identify the larvae up to the level of the genus of *Ancylostoma* sp.. The morphology of the infective third stage of *A. caninum*, described by Nichols (1955), is used. The morphology of the harvested larvae does not exactly coincide with *A. caninum* and comparable material is needed to properly distinguish different species, however there are no reasons for not classifying the harvested larvae as *Ancylostoma* sp.. Assuming the harvested larvae are *Ancylostoma* sp., a distinction between *A. caninum* and *A. tubaeforme* is questionable since *A. caninum* and *A. tubaeforme* are species-specific for dogs and cats respectively.[12] Furthermore, assuming the participating cats generally do not eat faeces of dogs, coprophagy and culturing passage eggs origin form dog faeces is not to be expected. Therefore the harvested larvae are probably *A. tubaeforme*. To adjust this diagnosis with certainty, molecular diagnostics of the larvae should be performed.

The questionnaire is answered by 177 participants for 338 individual cats. Seven participants did not answer the questionnaire for 16 individual cats. Two of these 16 cats are found positive for *Giardia*. Because of the lacking information about the lifestyle and deworming of the 16 cats, a complete picture of the results cannot be obtained.

Furthermore, one or more cats by each owner are registered for participating in this study. Cat owners with more than one cat have to separate the faeces of cats properly. One sample is needed of each individual cat. Some owners mentioned that it was difficult to distinguish the faeces of two or more cats in one household. Collected different faecal samples from the same cat, cannot be excluded. To make a correct interpretation of the faecal examinations, the samples have to be

collected, stored and transported correctly. Several participants did not store an transport the samples properly and it was unclear whether the samples were cooled before transporting. Without performing to the transport rules, eggs could continue to develop and are no longer recognizable or detectable with the conventional techniques, in case of *Ancylostoma* might hatch. and are no longer recognizable or detectable with the conventional techniques.[16] In this study, the most samples arrived within 2-3 days after sending. However, some samples were delayed by weekends and public holidays. Considering, the delayed samples have not been cooled for a longer period of time, the results of these samples are less reliable. In order to prevent a long sending time by weekends, samples should only be sent at the begin of the week.

Conclusion

The estimated prevalence of intestinal parasites in Dutch household cats in this study is 7,3%. 26 cats were found positive for intestinal parasites, of which 1 cat (0,3%) was found positive for *A. tubaeforme*. 191 cats (54%) were classified as indoor cats and 147 cats (41,5%) were classified as outdoor cats. From the indoor cats, nine cats were found positive. From the outdoor cats, 15 cats were found positive for intestinal parasites. It is unclear why the prevalence of all parasites is lower than reported in previous studies. The low prevalence of *A. tubaeforme* found in this study, made it impossible to study the association between the lifestyle of the cats and infection with *A. tubaeforme*. The association between the lifestyle of cats and infection with infection of intestinal parasites overall was statistical analyzed. It seems that outdoor cats have a higher prevalence (10,2%) of intestinal parasites than indoor cats (4,7%). Most of the cats were dewormed and the association between deworming and intestinal parasites was statistical analyzed. From the 13 non-dewormed cats, 1 cat was found positive for intestinal parasites. From the 325 dewormed cats, 23 cats were found positive for intestinal parasites. Dewormed cats have a significant higher prevalence of intestinal parasites than non-dewormed cats. Furthermore, the association between (0-4 times a year versus > 4 times a year) and lifestyle of the cats (indoor versus outdoor cats) is statistical analyzed. Significantly more outdoor cats are dewormed more than four times a year than indoor cats.

For further research, more cats need to be sampled for more reliability of the conclusions. Factors that influence the prevalence can be determined. The hookworm *A. tubaeforme* is less known worldwide. Research of the morphology of the larvae of *A. tubaeforme* is necessary for a complete determination and diagnose with certainty.

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Appendix I: Media call for participation

Voor een onderzoek van de faculteit Diergeneeskunde vragen wij het volgende:

KATTENPOEP GEZOCHT!

Heb jij of ken jij iemand met een kat en wil je een GRATIS onderzoek naar maagdarmwormen bij de kat laten uitvoeren? Dat is nu tijdelijk mogelijk.

Als studenten van de faculteit Diergeneeskunde aan de Universiteit Utrecht doen wij onderzoek naar het voorkomen van de haakworm *Ancylostoma tubaeforme* bij de kat. Natuurlijk kijken we ook of er andere darmparasieten in de ontlasting te vinden zijn.

Door mee te doen help je gelijk mee aan een onderzoek naar de noodzaak voor ontworming en krijg je bericht of jouw kat op dit moment eitjes uitscheidt en dus ontwormd moet worden.

Hoe werkt het?

- Wij zorgen voor materiaal zodat je de ontlasting op een veilige manier bij ons laboratorium kunt krijgen.
- Jij stuurt ontlasting op voorzien van de naam van het dier en een door ons gegeven code.
    - In het verzendpakket bevindt zich een retourenveloppe zodat dit kosteloos kan worden opgestuurd.
- Je krijgt een link voor een vragenlijst die je op jouw computer /smartphone kunt invullen.
- Als wij je vragenlijst hebben ontvangen en de uitslag is bekend, dan sturen wij jouw dit op, voorzien van een eventueel ontwormingsadvies.

Om tot een betrouwbaar resultaat te komen zijn veel katten nodig. Dit mogen dus binnenkatten, maar ook buitenkatten zijn.

Wil je meedoen? Stuur een mail naar parasietenwijzer@uu.nl onder vermelding van maagdarmwormonderzoek kat.

Mvg,

Sandra Vink en Manon Coenen

Appendix II: Affiche for participation

Universiteit Utrecht

KATTENPOEP GEZOCHT!

Heeft u of kent u iemand met een kat en wilt u een **GRATIS onderzoek naar maagdarmwormen** bij de kat laten uitvoeren? Dat is nu tijdelijk mogelijk.

Als student van de faculteit Diergeneeskunde aan de Universiteit Utrecht doe ik onderzoek naar het voorkomen van de haakworm *Ancylostoma tubaeforme* bij de kat. Natuurlijk wordt er ook gekeken of er andere darmparasieten in de ontlasting te vinden zijn.

Door mee te doen helpt u gelijk mee aan een onderzoek naar de noodzaak voor ontworming en krijgt u bericht of uw kat op dit moment eitjes uitscheidt en dus ontwormd moet worden.

Er zijn zoveel mogelijk katten nodig!

Wilt u meedoen? Meldt u dan nu aan en geef uw emailadres op, zodat ik contact met u kan opnemen.

Sandra Vink

Naam	**Emailadres**
1.	
2.	
3.	
4.	
5.	
6.	
7.	
8.	
9.	
10.	
11.	
12.	
13.	
14.	
15.	

Appendix III: Package instructions

Geachte heer/mevrouw,

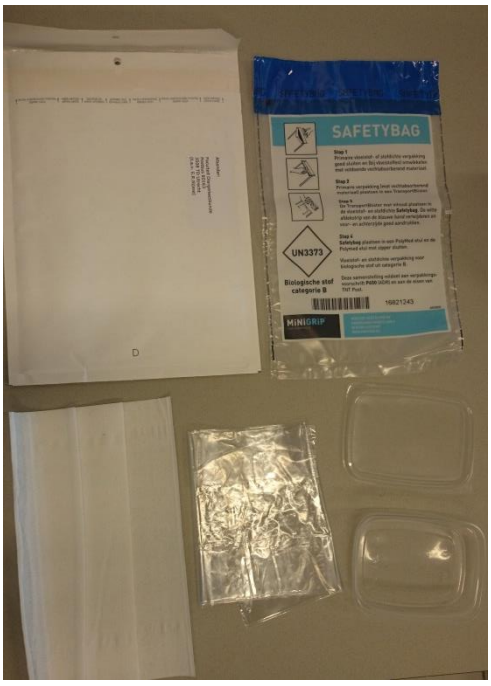
Bedankt voor uw deelname aan ons onderzoek.

Hierbij ontvangt u het deelname pakket en vindt u per kat het volgende:

1. Een plastic zakje om ontlasting in te doen
2. Een plastic bakje met deksel
3. Tissues
4. Een sealbag (per 2 katten 1 sealbag)
5. Een gewatteerde verzendenveloppe

In één gewatteerde verzendenveloppe passen 3 monsters, deelnemers met meerdere katten kunnen dus 3 monsters van verschillende katten in één enveloppe verzenden.

Figuur 1: De inhoud van het pakket.



Uw persoonlijke deelnamenummer tijdens dit onderzoek:

Gelieve dit nummer ergens te noteren. Dit nummer blijft gedurende het hele onderzoek gelijk en dient u te noteren op de deksel van het bakje waar de ontlasting in gaat. Indien u met meerdere katten mee doet, dient u dit nummer op elk dekseltje te vermelden. Dit nummer dient u ook bij de enquête in te vullen. Wanneer u vragen stelt via de mail kunt u hier ook dit nummer in het onderwerp vermelden.

Uitleg over de monstername:

De ontlasting dient u in het plastic zakje te doen. Zorg ervoor dat er geen/zo min mogelijk ander materiaal in komt, zoals kattengrit. Knoop het zakje vervolgens dicht met zo min mogelijk lucht er in. Indien u niet in de gelegenheid bent om het pakketje direct op te sturen, adviseren wij de ingepakte ontlasting tijdelijk (niet langer dan één dag) koel te bewaren.

Uitleg opsturen monster: (zie fotoserie aan het einde)

1. Het zakje met de ontlasting stopt u in het plastic bakje met de deksel erop
2. Schrijf de naam van de kat, het deelnamenummer en de datum van verzamelen op de deksel
3. Het plastic bakje wikkelt u in een tissue
4. Stop dit in zijn geheel in de sealbag, plak deze met de zelfklevende rand dicht met zo min mogelijk lucht erin.
5. De sealbag gaat in de envelop

Deze stuurt u naar het volgende adres (envelop is al gestickerd):

Faculteit Diergeneeskunde
VMDC (wormonderzoek kat)
Antwoordnummer 57526
3507 WB Utrecht

Hier hoeft dus géén postzegel op!

Uitleg enquête:

1. Wanneer de ontlasting bij ons binnenkomt, krijgt u van ons een e-mail met bevestiging van ontvangst.
2. Via deze e-mail ontvangt u ook de link naar de enquête.
3. Volg de stappen van de enquête.
4. De enquête zal ongeveer 10 minuten van uw tijd in beslag nemen.
5. De enquête wordt, wanneer deze volledig is ingevuld, automatisch naar ons gestuurd.
6. Pas als de ingevulde enquête bij ons binnen is, krijgt u uitslag van het onderzoek met een eventueel ontwormingsadvies.

Wij willen u vragen om ons via een mailtje te laten weten dat u het pakketje heeft opgestuurd.
In slechts een enkel geval kan het voorkomen dat een pakketje tijdens het vervoer met de post zoek raakt. Op deze manier kunnen wij dit in de gaten houden.

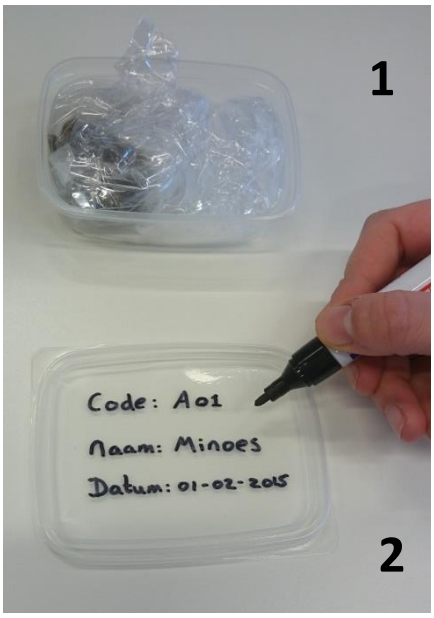
Bij vragen kunt u te allen tijde contact met ons opnemen via onderstaand e-mailadres o.v.v. Uw deelnamenummer en “Maagdarmwormonderzoek kat”:

parasietenwijzer@uu.nl

Met vriendelijke groet,

Sandra Vink en Manon Coenen

Gebruik van het pakket:



Appendix IV: Questionnaire

Dank u wel voor uw deelname aan dit onderzoek.
De informatie die u verstrekt, wordt vertrouwelijk behandeld en alleen gebruikt voor dit onderzoek.
De uitslagen worden losgekoppeld van uw persoonlijke gegevens.

*Op een vraag met een * is een antwoord noodzakelijk om verder te kunnen gaan met het invullen van de enquête. Er zijn geen goede/foute antwoorden.*

Het invullen van de enquête zal afhankelijk van het aantal katten gemiddeld 10-15 minuten in beslag nemen, deze tijd is niet af te lezen aan de voortgangsbalk bovenin.

LET OP: Pak voor het invullen van deze enquête zo mogelijk de verpakking van uw ontwormingsmiddel en/of ontvlooiingsmiddel erbij.

Gegevens kat
Allereerst vragen wij u de gegevens van uw kat(ten) in te vullen.
Deze gegevens zullen alleen voor de identificatie tijdens dit onderzoek gebruikt worden.
∗1. Voer hieronder uw persoonlijke deelnamenummer (code) in.
A [ ][ ][ ]

Gegevens van uw kat
∗2. Vul hieronder de gegevens van uw enige/eerste kat in.
Als het ras en/of geslacht niet bekend zijn, vult u hier dan "onbekend" in. De naam is wel verplicht ter identificatie.
Als u met meerdere katten mee wilt doen aan dit onderzoek, komen deze later aan bod. De volgorde van de verschillende katten is niet van belang.
Aantal katten: [ ]
Naam van de (enige) deelnemende kat: [ ]
Ras: [ ]

∗3. Wat is het geslacht van deze kat?
- o Poes gesteriliseerd
- o Poes niet gesteriliseerd
- o Kater gecastreerd
- o Kater niet gecastreerd
- o Onbekend

Gegevens van uw kat
∗4. Vul hieronder de leeftijd van uw kat in, het liefst met een geboortedatum.
- o Jonger dan 6 maanden
- o 6 maanden tot 1 jaar
- o 1 jaar
- o 2 jaar
- o 3 jaar
- o 4 jaar
- o 5 jaar
- o 6 jaar
- o 7 jaar
- o 8 jaar

- 9 jaar
- 10 jaar
- 11 jaar
- 12 jaar
- 13 jaar
- 14 jaar
- Ouder dan 14 jaar

Geboortedatum (dd/mm/jjjj) (als niet bekend, vul dan "onbekend" in): [ ]

Ontwormingsschema

∗5. Is uw kat wel eens ontwormd?

- Ja
- Nee

Motivatie ontwormingsschema

∗6. Wat is de belangrijkste reden om een kat in het algemeen te ontwormen?

- De gezondheid van de kat te waarborgen
- De gezondheid van de mens te waarborgen
- Omdat het moet

∗7. Wat is uw motivatie om uw kat te ontwormen? (er zijn meerdere antwoorden mogelijk)

- Na een ontlastingsonderzoek
- Op advies van de dierenarts
- Op advies van de dierenspeciaalzaak
- Op advies van een kennis
- Door informatie van het internet
- Door informatie vanuit mijn opleiding of werk
- Ik heb wormen/eipakketjes gevonden in de kattenbak/mandje van de kat
- Anders, namelijk [ ]

∗8. Welk ontwormingsschema zou u graag voor deze kat willen aanhouden?

- Geen voorkeur
- 1 maal per jaar
- 2-3 maal per jaar
- 4 maal per jaar
- > 4 maal per jaar, namelijk (aantal invullen): [ ]

∗9. Welk ontwormingsschema houdt u voor deze kat aan?

- Ik houd geen ontwormingsschema aan
- 1 maal per jaar
- 2-3maalperjaar
- 4 maal per jaar
- > 4 maal per jaar, namelijk (aantal invullen): [ ]

∗10. Op basis van welke informatie(bron) heeft u het ontwormingsschema gebaseerd?

- Op advies van de fokker
- Op advies van de dierenarts
- Op advies van de dierenspeciaalzaak
- Op advies verkregen via internet
- Op advies verkregen via mijn opleiding of werk
- Anders, namelijk

Informatiebron

∗11. Welke website heeft u als informatiebron gebruikt?

- o Een site van een dierenartsenpraktijk
- o Een kattenforum
- o Een andere site, namelijk: [ ]

12. Hoe lang denkt u dat een ontwormingsmiddel werkzaam is?

- o Kort tot enkele dagen
- o 1 maand
- o 2-3 maanden
- o 1 jaar
- o Weet ik niet

Ontwormen

13. Wanneer is uw kat voor het laatst ontwormd?

- o Minder dan 1 week geleden
- o 1 - 2 weken geleden
- o 2 weken - 1 maand geleden
- o 1 - 2 maanden geleden
- o 2 - 3 maanden geleden
- o 3 - 4 maanden geleden
- o 5 - 6 maanden geleden
- o Langer dan 6 maanden geleden

∗14. Met welk middel is uw kat voor het laatst ontwormd?

Indien onbekend "onbekend" invullen.

Naam ontwormingsmiddel: [ ]

∗15. Gebruikt u dit ontwormingsmiddel doorgaans ook?

- o Ja
- o Nee

∗16. Met welk middel wordt uw kat doorgaans ontwormd?

Indien onbekend "onbekend" invullen.

Naam ontwormingsmiddel: [ ]

Ontwormen

∗17. Waar koopt u uw ontwormingsmiddelen? (er zijn meerdere antwoorden mogelijk)

- o Dierenarts
- o Dierenspeciaalzaak
- o Supermarkt/warenhuis/tuincentrum
- o Internet

∗18. Wat is de toedieningsvorm van het ontwormingssmiddel? (er zijn meerdere antwoorden mogelijk)

- o Tablet
- o Pasta/drankje
- o Spot-on (druppels op de huid)
- o Anders, namelijk

Ontwormingsschema

∗19. Vindt de ontworming samen met de jaarlijkse vaccinatie bij de dierenarts plaats?

- Ja
- Nee

Ontwormen

∗20. Waarom ontwormt u uw kat niet? (er zijn meerdere antwoorden mogelijk)

- Ik vind het niet nodig
- Ik vind het te duur
- Ik vind het te veel moeite
- Ik heb hier nooit over nagedacht
- Mijn kat komt niet buiten
- Anders, namelijk [ ]

Gegevens van uw kat

∗21. Komt uw kat buiten?

- Ja
- Nee

∗22. Hoe omschrijft u de omgeving waarin de kat buiten loopt? (er zijn meerdere antwoorden mogelijk)

- Woonwijk
- Bosrijk
- Zee-/duingebied
- Platteland
- Balkon
- Afgeschermde tuin

∗23. Kunnen andere katten dan die van uzelf hier ook komen?

- Ja
- Nee

Schoeisel binnen

∗24. Loopt u met uw buitenschoenen in huis?

- Ja
- Nee

Samenstelling huishouden

∗25. Heeft u naast uw kat(ten) nog andere huisdieren?

- Nee
- Ja, een of meerdere honden
- Ja, anders [ ]

∗26. Zijn er in uw huishouden kinderen jonger dan 16 jaar?

- Ja
- Nee

27. Wat is de leeftijd van uw jongste kind?

Leeftijd: [ ]

28. Wordt uw hond ontwormd?

- Ja
- Nee

Voeding van de kat

∗29. Mijn kat eet (wel eens).... (er zijn meerdere antwoorden mogelijk)

- Rauw vlees
- Rauwe vis
- Voorverpakt droogvoer/blikvoer
- Prooidieren
- Met de pot mee
- Anders, namelijk [ ]

∗30. Welke prooidieren worden voornamelijk gegeten door uw kat? (er zijn meerdere antwoorden mogelijk)

- Vogel
- Muis/Rat
- Kikker/Pad
- Konijn
- Vis
- Insecten (vlieg, libelle, ect.)
- Gevonden dode dieren
- Andere prooidieren
- Weet ik niet

∗31. Wat voor soort rauw vlees eet uw kat voornamelijk? (er zijn meerdere antwoorden mogelijk)

- Rund
- Varken
- Paard
- Schaap
- Konijn
- Kip
- Ander gevogelte

∗32. Wat is de herkomst van het rauwe vlees? (er zijn meerdere antwoorden mogelijk)

- Supermarkt
- Slagerij / poelier
- Eigen fok
- Slachthuis
- Via internet

Kennel- of pensionverblijf

∗33. Heeft uw kat de afgelopen 6 maanden in een asiel of pension gezeten?

- Ja
- Nee

Aanwijzingen verminderde gezondheid

∗34. Zijn er bij uw kat aanwijzingen voor een verminderde gezondheid? Denk hierbij aan: diarree, jeuk, benauwdheid etc.

- Ja
- Nee

Aanwijzingen verminderde gezondheid

∗35. Welke aanwijzingen voor een verminderde gezondheid zijn er gevonden bij uw kat?

[ ]

Medicijngebruik

∗36. Krijgt uw kat op dit moment één of meerdere medicijnen toegediend?

- ○ Ja
- ○ Nee

Medicijngebruik

∗37. Welke medicijnen krijgt uw kat op dit moment toegediend?

Bij voorkeur middel en merknaam. Indien onbekend: "onbekend" invullen

1: [ ]
2: [ ]

Medicijngebruik

∗38. Heeft uw kat de afgelopen 3 maanden medicijnen toegediend gekregen?

- ○ Ja
- ○ Nee

Medicijngebruik

∗39. Heeft uw kat in de laatste 3 maanden nog ANDERE medicijnen toegediend gekregen?

- ○ Ja
- ○ Nee

Medicijngebruik

∗40. Welke medicijnen heeft uw kat in de afgelopen 3 maanden toegediend gekregen?

Bij voorkeur middel en merknaam.
Als beide onbekend zijn: "onbekend" invullen

1: [ ]
2: [ ]

Ontlasting

∗41. Waar doet uw kat doorgaans zijn/haar behoeften?

- ○ Op de kattenbak
- ○ Buiten
- ○ Zowel op de kattenbak als buiten
- ○ Anders, namelijk [ ]

Ontlasting

∗42. Hoe vaak schept u vieze delen uit de kattenbak?

- ○ Meer dan 3 keer per week
- ○ 2-3 keer per week
- ○ 1 keer per week
- ○ Minder dan 1 keer per week

∗43. Hoe vaak verschoont u de gehele vulling van de kattenbak?

- ○ Meer dan 3 keer per week
- ○ 2-3 keer per week

- 1 keer per week
- Minder dan 1 keer per week

Ontlasting

∗44. Is u de laatste tijd nog iets opgevallen aan de ontlasting van uw kat? (er zijn meerdere antwoorden mogelijk)

- Nee
- Ja, het is dunner
- Ja, ik heb wormen/eipakketjes gevonden **gfedc** Ja, het is donkerder
- Ja, het is lichter
- Ja, er zat bloed bij
- Ja, er zat slijm bij
- Anders, namelijk

∗45. Welke worm/eipakketten denkt u te hebben gevonden?

- Spoelworm
- Lintworm
- Haakworm
- Zweepworm
- Weet ik niet

∗46. Zijn de worm/eipakketten door een dierenarts bevestigd?

- Ja
- Nee

Giardia

∗47. Is uw kat, voor zover u weet, ooit besmet geweest met Giardia?

- Ja
- Nee

∗48. Wanneer was de besmetting met Giardia?

[ ]

∗49. Had de kat toen klachten?

- Nee
- Ja, diarree
- Ja, buikpijn
- Ja, braken
- Ja, doffe vacht
- Ja, anders, namelijk [ ]

∗50. Is er vervolgens een behandeling ingesteld?

- Ja
- Nee
- Weet ik niet

∗51. Welke behandeling is er ingesteld?

- Voerwisseling
- Kat gewassen
- Omgeving gereinigd

- Medicijnen
- Anders, namelijk [ ]

∗52. Weet u welke medicijnen er zijn voorgeschreven?

- Nee
- Ja, namelijk [ ]

∗53. Zijn de klachten verdwenen?

- Ja
- Nee
- Weet ik niet

∗54. Is de Giardia infectie verdwenen?

- Ja
- Nee
- Niet gecontroleerd

Deelname meerdere katten

∗55. Heeft u nog meer katten opgegeven voor deelname aan dit onderzoek?

- Ja
- Nee

Gegevens van uw kat

∗56. Vul hieronder de gegevens van uw tweede kat in.

Als het ras en/of geslacht niet bekend zijn, vult u hier dan "onbekend" in. De naam is wel verplicht ter identificatie.
Als u met meerdere katten mee wilt doen aan dit onderzoek, komen deze later aan bod. De volgorde van de verschillende katten is niet van belang.

Naam van de tweede deelnemende kat: [ ]
Ras: [ ]

Etcetera…
The same questions were asked again if the owner particpate with more than one cat (total of five cats).

Appendix V: Protocol CSF

1. Prepare a suspension of the feces sample in water. If the feces is too solid, use a morter.

2. Pour the suspension over a strainer.

3. Stir the strained suspension before filling a test-tube (the eggs lie at the bottom). Place the test-tube inside the centrifuge. Make sure that two equally filled test-tubes always oppose each other.

4. Close the lid of the centrifuge and turn it on.

5. Keep the centrifuge running for 1 minute at 3000 rpm.

6. Wait until the centrifuge has completely stopped and open the lid.

7. Pour the supernatant out of the test-tube with a slow turning motion.

8. Fill the test-tube for about 50% with a sucrose suspension. Mix the sediment with a small spatula.

9. Place the test-tube back inside the centrifuge and fill the test-tube complete with sucrose suspension until a small meniscus forms.

10. Place a cover glass on top of the meniscus. Press the cover glass firmly onto the testtube with a nail.

11. Keep the centrifuge running for 2 minutes at 3000 rpm.

12. Take off the cover glass perpendicular from the test-tube and put it on an object glass.

13. Systematically search the preparation for worm eggs.

Appendix VI: Protocol Baermann technique

1. Place a strainer in a Baermann glass.

2. Fill the glass with tap water until the strainer is 50% filled.

3. Place the feces that needs to be analyzed in the strainer filled with water.

4. Leave the Baermann standing for 24 hours at room temperature.

Checking the Baermann after 24 hours:

5. Put a small balloon on a pipette.

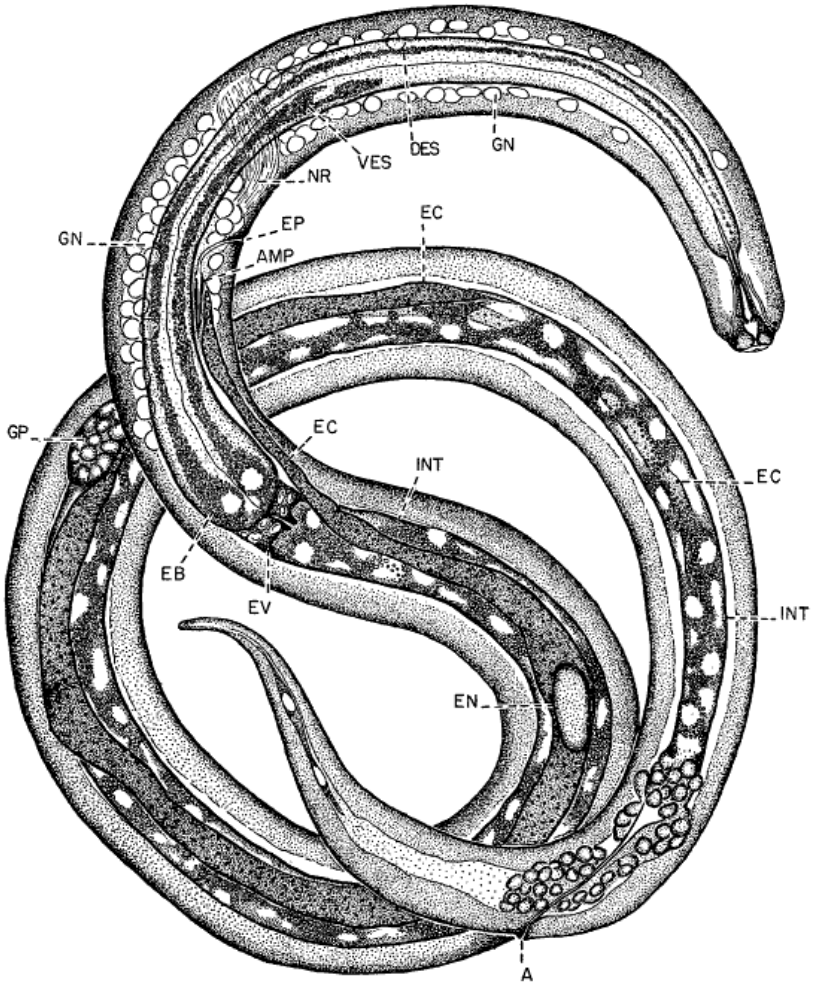
6. Take the strainer out of the glass and put the pipette in the glass all the way to the tip with the small balloon squeezed between two fingers.

7. Let go of the small balloon so that the pipette can fill with fluid.

8. Take the pipette out of the glass and squeeze it empty into a small Petridis.

9. Place the small Petridish under a preparation microscope and systematically search for worm larvae.

Appendix VII: Drawings of infective third stage larvae of *Ancylostoma caninum* (Heat-killed and unstained)



GN = Glandular esophagus
DES = Dorsal esophageal gland
VES = Ventral esophageal gland
NR = Nerve ring
EP = Excretory pore
AMP = Excretory ampulla
EB = Esophagal bulb
EC = Excretory columns
EV = esophago intestinal valve
INT = Intestine
EN = nucleus of excretory cell
GP = Genital primordium
A = Anus

Appendix VIII: Dutch map of the distritbution of the participants

Appendix IX: Crosstabs and statistical analysis

A: Correlation between the prevalence of intestinal parasites and lifestyle

Chi-square Frequencies

Lifestyle

	Observed N	Expected N	Residual
Indoor cat	191	169,0	22,0
Outdoor cat	147	169,0	-22,0
Total	338		

Parasites

	Observed N	Expected N	Residual
Negative for intestinal parasites	314	169,0	145,0
Positive for intestinal parasites	24	169,0	-145,0
Total	338		

Test Statistics

	Lifestyle	Parasites
Chi-Square	5,728[a]	248,817[a]
df	1	1
Asymp. Sig.	,017	,000

a. 0 cells (,0%) have expected frequencies less than 5. The minimum expected cell frequency is 169,0.

B: Correlation between the prevalence of intestinal parasites and deworming

Chi-square Frequencies

Deworming

	Observed N	Expected N	Residual
Not dewormed	13	169,0	-156,0
Dewormed	325	169,0	156,0
Total	338		

Parasites

	Observed N	Expected N	Residual
Negative for intestinal parasites	314	169,0	145,0
Positive for intestinal parasites	24	169,0	-145,0
Total	338		

Test Statistics

	Deworming	Parasites
Chi-Square	288,000[a]	248,817[a]
df	1	1
Asymp. Sig.	,000	,000

a. 0 cells (,0%) have expected frequencies less than 5. The minimum expected cell frequency is 169,0.

C: Correlation between deworming (0-4 times a year versus > 4 times a year) and lifestyle (indoor versus outdoor cats)

Chi-square Frequencies

Dewormschedule

	Observed N	Expected N	Residual
0-4 times a year	330	169,0	161,0
> 4 times a year	8	169,0	-161,0
Total	338		

Lifestyle

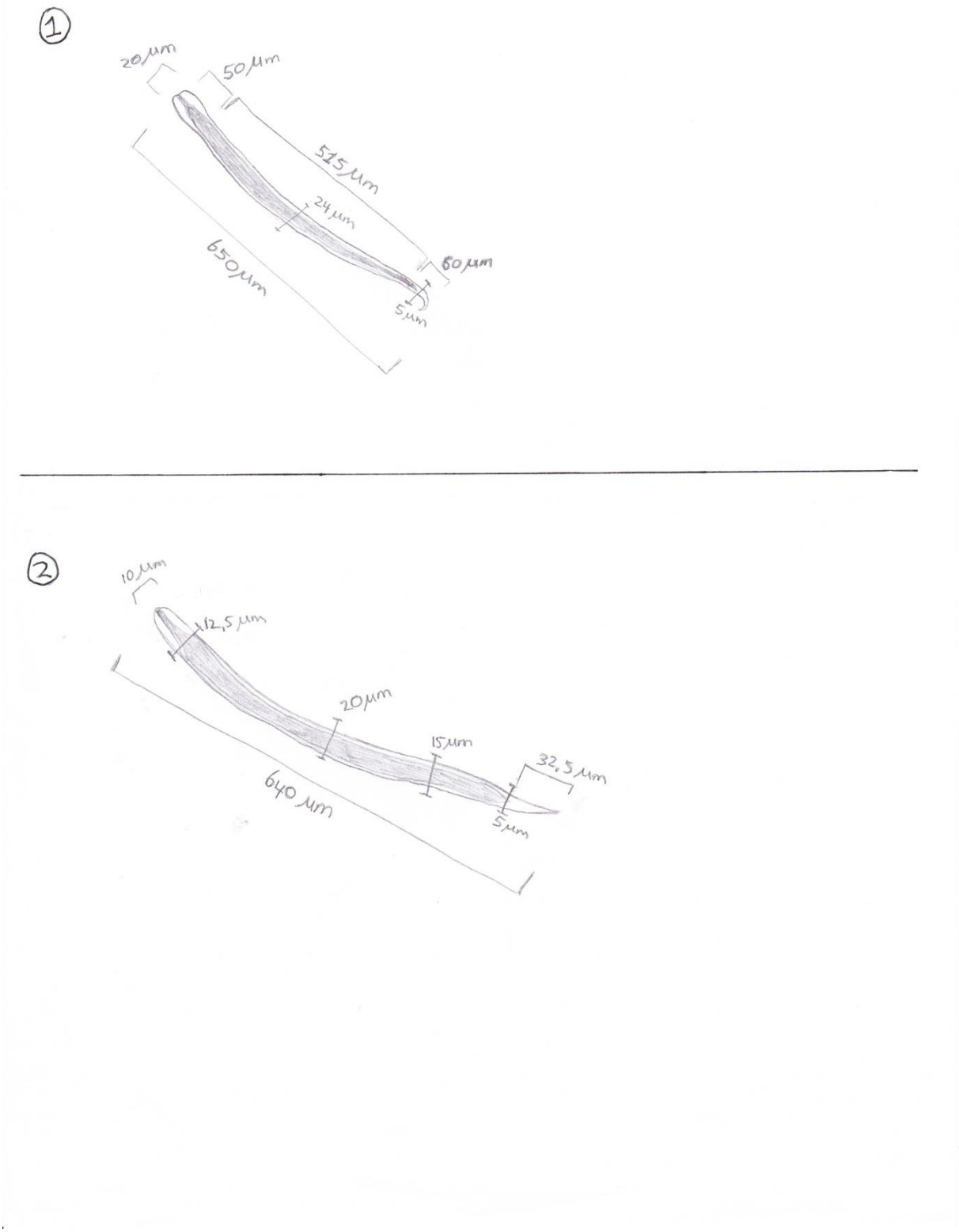
	Observed N	Expected N	Residual
Indoor cat	191	169,0	22,0
Outdoor cat	147	169,0	-22,0
Total	338		

Test Statistics

	Dewormschedule	Lifestyle
Chi-Square	306,757[a]	5,728[a]
df	1	1
Asymp. Sig.	,000	,017

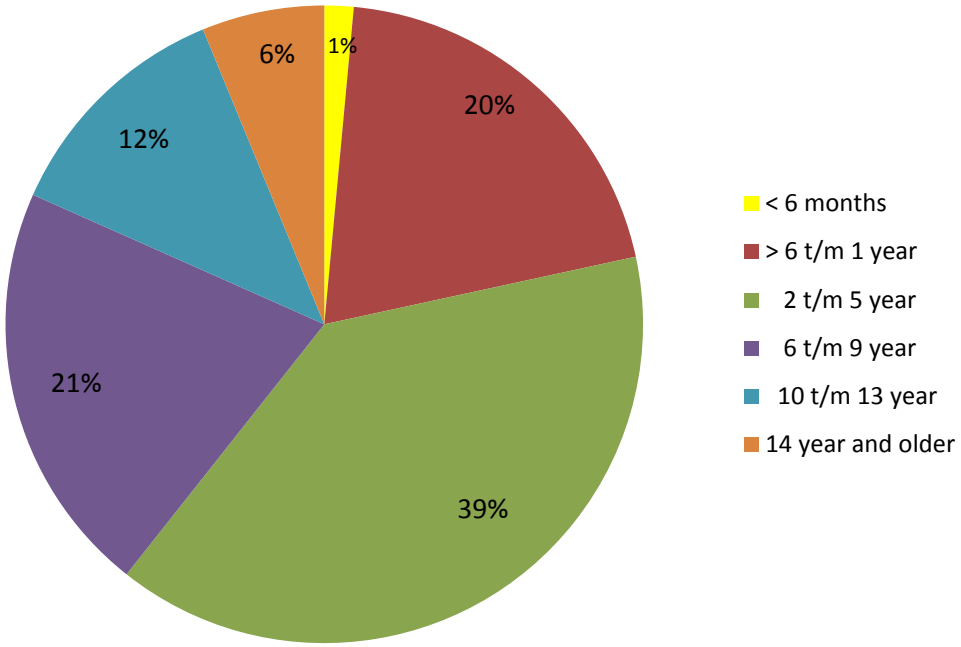
a. 0 cells (,0%) have expected frequencies less than 5. The minimum expected cell frequency is 169,0.

Appendix X: Drawings of the harvested, fixed larvae

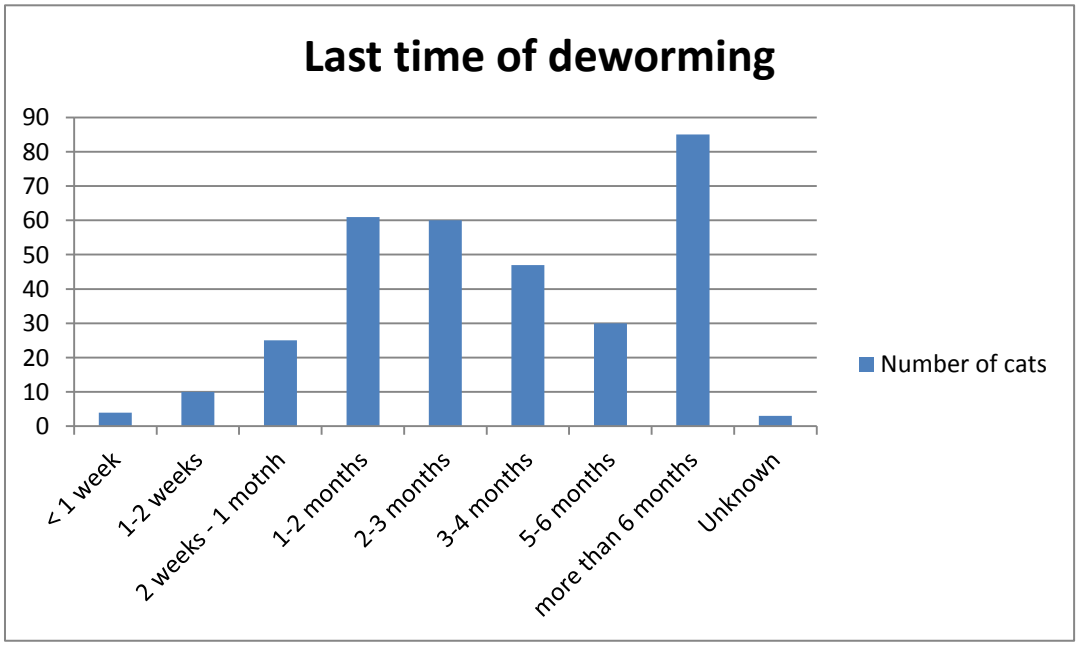


Appendix XI: Charts on descriptive statistics

A: Age of individual participating cats (without the unknown group)



B: Last time of deworming



C: Deworming schedule compared with indoor and outdoor cats in percentage