**The relationship between a raw meat diet for dogs and a patent infection with *Toxocara canis* of dogs in the Netherlands.**

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**Abstract**

There are several risks for the occurrence of a patent *T.canis* infection, for example demographic data, behaviour of the dog, circumstances the dog lives in and the health status of the dog. In this study we focused on the possible risk of feeding raw meat and the development of a patent infection with *T.canis* in the dog. In paratenic hosts, ingestion of *T.canis* eggs leads to somatic migration and encapsulated larvae in the tissue. When this meat with encapsulated larvae is eaten without cooking by a dog these larvae could lead to a patent infection.

The purpose of this study is to investigate the association between dogs eating raw meat and a patent *T.canis* infection.

Dog owners in the Netherlands were asked to participate voluntarily in this research for two years. The owners of the participating dogs collected faecal samples every month and send them to the Faculty of Veterinary Science of the University of Utrecht. The samples were processed according to the centrifuge sedimentation flotation method with sucrose (1,27-1,29 kg/dm3) as flotation medium. The slides were examined microscopically for *T.canis* eggs.

Also a questionnaire was send via the e-mail to participants to gather information about possible risk factors that could cause a patent *T.canis* infection. Because this research focussed on raw meat, an additional questionnaire was send to owners of dogs that fed their dog(s) raw meat with questions about the origin of the meat and factors of risk associated with the meat.

A significant association was found between dogs that did not eat raw meat and a patent *T.canis* infection. We also found a significant association between dogs not at risk for a patent *T.canis* infection, which included dogs that ate raw meat from intensively kept meat producing animals or commercially available dry and canned dog food, and a patent *T.canis* infection.

No association was found between dogs eating raw meat and a patent *T.canis* infection.

**Introduction**

*Toxocara canis* is a common large roundworm of dogs. Males can become 10 cm and females up to 18 cm in length.1 This nematode lives in the lumen of the small intestine of the dog and feeds on the contents of it. 2 According to a study in 2009, in 4,4% of clinically healthy dogs, visiting a vet in the Netherlands, *T.canis* eggs were found in the faeces.3  Female worms in the intestine of the dog produce 200.000 eggs a day. 1, 2 These *T.canis* eggs are unembryonated (L1 stage4) when they are shed and are not infective. Depending on the environmental circumstances, eggs can develop into infective L3 stage larvae in two weeks to several months. These infective embryonated eggs can survive in the environment with optimal conditions for at least one year. 4, 5

For *Canidae* there are four routes of infection:

* Ingestion of embryonated, L3 stage, eggs from the environment.
* Eating a paratenic host with encapsulated L3 larvae.
* The transplacental route, which is the most important route for dogs.
* The lactogenic route, which is less important for *Canidae*. 1, 2, 4

Foxes are, just like dogs, the final host of *T.canis*. In a study of Reperant et al. a percentage of 44,3% of the foxes in Geneva, Switzerland, were found *T.canis* positive. 27 In the Netherlands in 1984 even 73,3% of the foxes had *T.canis* larvae and worms in their intestine. 28 So there has to be something in the lifestyle of foxes and dogs that predisposes to a patent infection.

In pups till 2-3 months of age, infection with *T.canis* leads to a tracheal migration. After ingestion larvae penetrate the intestinal mucosa and travel via the mesenterial lymph nodes to the liver. Then they travel to the lungs where they penetrate the alveoli and migrate via the trachea to the pharynx where they are swallowed and reach the intestine. In the intestine of the dog they grow from a L5 larval stage into egg producing adult worms, which results in a patent infection. 1, 2, 4, 6 A patent infection means that the parasite that is infecting the tissues of an animal is productive, thus producing eggs in case of *T.canis*.7 In an infection, the parasite causes damage to the host but is not productive.8 In the lifecycle of *T.canis* the shedding of eggs by the dog is important for the continuation of the lifecycle of the parasite.

In dogs from 3 to 6 months of age there will be more somatic migration and from 6 months on there will be mostly somatic migration. The reason for this change in route of migration is the development of immunity against *T.canis* larvae in the lungs and the gastro-intestinal tract. During this somatic migration, the larvae do not travel via the lungs but are redistributed to other tissues where they become encapsulated. 1, 2, 4, 6

Especially pups show signs of a *T.canis* infection that include tachypnea and coughing due to tracheal migration. Other signs include diarrhoea, pot-bellied appearance, delay in growth and losing weight. A lot of adult worms are able to cause a partial of total obstruction of the intestine which sometimes leads to perforation and peritonitis. If there is a massive infection, pups can die in a few days.1, 2, 4, 6 Infection in adult dogs will seldom lead to clinical signs. In case of ingestion of very large quantities of infective *T.canis* eggs diarrhoea could be a symptom of the adult dog.2

One specific route of infection for the dog is eating paratenic hosts.1, 2, 5 A paratenic host is an animal that serves as an intermediate host which is not a necessary step in the cycle of the parasite and in which the parasite will not develop itself. Its purpose in the lifecycle of *T.canis* is to transmit the parasite to a next suitable final host by which it has to be eaten.9 *T.canis* eggs will be ingested by the paratenic host via the environment followed by a somatic migration. The larvae become encapsulated in the liver during their migration and in the muscle-10, kidney-11, 12, spleen-11 or brain13 tissue it is distributed to, depending on the animal species.14 The larvae go in hypobiosis and will not develop until they are eaten by a final host. 14 In the mean time they will not be killed by the hosts immune system because of, among others, an antigenic coat. The larvae develop Excretory/Secretory products that cover the entire surface of the larvae. When antibodies attach, *T.canis* can lose its coat, including the antibodies, and prevent an immune response which would normally eliminate the parasite. 4

Subsequently the paratenic host, or the meat of the paratenic host, will be eaten by the dog and larvae develop directly into adult worms in the intestine without tracheal migration. There will be no migration, probably because the larvae already have gone through the somatic migration in the paratenic host so it is not necessary to perform another somatic migration in the dog. 1, 6

Thereby the adult dog has developed immunity specific for L3 infective *T.canis* larvae in the intestine which prevents larvae to penetrate the intestinal wall and prevents them from growing into adult worms.6 However, this is not in line with the possibility of L3 larvae leading to a patent infection and a somatic migration. It is unclear how the immune system of dogs exactly affects the larvae in the gastro-intestinal tract.

Paratenic hosts are animals that can ingest infective *T.canis* eggs from contaminated soil, this includes animals used for meat production. Dogs fed raw meat of these animals, can become patent infected with *T.canis*. In the 21th century pet owners became more interested in an alternative way of feeding for commercially available dog food, and raw meat diets became more popular. Mostly because of distrust against commercial dog food, specific medical demands for their pet and involvement in choosing the ingredient for their own dog’s meal.15 Nowadays there are several different ways to feed raw meat to the dog. There is raw meat that is commercially available as a complete pet food. In the Netherlands it is called KVV which stands for ‘complete fresh food’. There are also several ways to prepare a self-made raw meat diet. These ways of raw feeding are called BARF, NRV, the Ultimate diet and the Volhard diet.16 BARF stands for ‘Biologically Appropriate Raw Food’ or ‘Bones And Raw Food’ and the diet contains 60% raw meat with bones, supplemented with products that a dog in the wild would eat.17 NRV stands for ‘Natural Raw Food’ and is based on the principle of a dog eating prey. The diet contains no supplements, just complete carcasses.16 The Ultimate diet is based on a pyramid which contains raw meat in the bottom layer of the pyramid, followed by raw vegetables, and the top of the pyramid consists of supplement nutrients that aren’t sufficient available in the bottom layers.18 Finally there is the Volhard diet which consists of a mixture of grains and yoghurt in the morning and raw meat, vegetables and fruit in the evening.19 Because of the discussed life cycle of *T.canis* in paratenic hosts, these ways of feeding dogs could cause a higher risk of developing a patent infection.

Preparation of meat at temperatures of 70-78°C will inactivate all larvae. That is why prepared meat is not at risk for giving a patent infection in dogs and raw meat is.20, 21 Also temperatures of -25°C and less kill the larvae in the meat but unfortunately this temperature will not be reached in an ordinary freezer where the temperature is usually -18°C. Temperatures between these ranges don’t kill the larvae so they will stay infective.22 There is no study available about the effect of -18°C for a longer period of time on the *T.canis* eggs encapsulated in the meat.

Besides dogs, people can also get infected with infective *T.canis* eggs while they serve as a paratenic host. Ingestion of infective *T.canis* eggs via contaminated soil or vegetables leads to a somatic migration.23, 24 Dogs shedding T.canis eggs into the environment serve as a factor of risk for human toxocarosis. When a lot of *T.canis* larvae invade the tissues of the human, there will be an immune reaction that leads to Visceral Larva Migrans syndrome (VLM). Most larvae die or become encapsulated. Clinical signs are most common in children of 1 to 7 years of age because they are at higher risk of contact with contaminated soil in playgrounds for example. Clinical signs include fever, abdominal pain, tachypnea and coughing. When there are less *T.canis* larvae, migrating larvae can reach the retina and cause Ocular Larva Migrans syndrome (OLM). This syndrome shows signs of loss of vision, seeing lights and is usually seen amongst children of 8 years. There is a third form, the Covert Toxocarosis. This syndrome consists of clinical symptoms that are not specific for VLM or OLM but are a vague complex of symptoms like coughing, headache, abdominal pain and behavioural changes. Adults usually show no signs of toxocarosis.5, 23, 24 Research of Pinelli et al. showed that a *T.canis* infection can lead to exacerbation of airway infections and it contributes to allergies like asthma. There is found a significant association between the occurrence of asthma/recurrent bronchitis and *Toxocara* seropositivity.25

According to the lifecycle of *T.canis*, dogs eating raw meat should be at higher risk for a patent *T.canis* infection. But it is important to know the origin of the meat because animals kept in closed stables are unlikely to become infected with *T.canis* so there will no larvae be encapsulated in tissues. Free ranging meat producing animals however, are at greater risk of becoming infected with infective *T.canis* eggs from contaminated soil when searching for food. When dogs eat meat of these infected paratenic hosts, they could develop a patent infection and shed *T.canis* eggs in the environment. With a supplementary online survey questions are asked to owners, who feed their dog(s) raw meat, about the origin of the meat. This way we want to find out more about the lifecycle of *T.canis* in the paratenic host and the risk for consumption of raw meat by the final host. Because of the zoonotic risk of *T.canis* it is important to know if raw meat gives a patent infection in dogs. In this article the role of the raw meat diet for the dog in a patent *T.canis* infection will be investigated.

**Materials and methods**

Faecal samples

Recruitment of participants in Utrecht and Den Haag started in 2011 via flyers in dog walking areas (forest, parks, beaches etc.), pet stores and vets, social media, internet fora etc. They were asked to participate in an independent research at the Faculty of Veterinary Medicine at the University of Utrecht about patent infections of the roundworm *Toxocara canis* in dogs*.* Through dog breeders and social media people all over the Netherlands were reached. People had to e-mail their name and address to parasietenwijzer@uu.nl when they wanted to participate in this free and voluntarily research. Faecal samples had to be send in every month for two years and participating dogs should be older than 6 months of age. Eventually there were about 632 participants with a total of 905 dogs from which faecal samples were send to the laboratory for coproscopical examination. Because of the large number of participants the recruitment of new participants stopped in August 2012. The coproscopical examination of dogs of the assembled participants for this research, continues till 2014.

Every participant received an unique number used to link, together with the dogs name, the participating dog with the faecal sample that is send in. Also the results are saved together with this number so all the information from the participant is coupled with this unique number. Participants also receive materials to collect and send the faeces safely to the lab. Instructions are added that tell the participants how to pack and send the faecal monsters.

When envelopes with faecal samples are received at the laboratory, the unique numbers of the participants are registered on the weekly laboratory list. All the incoming faecal samples receive a laboratory number starting with number 1 on Mondays and the last number ends on Friday. They are written in the laboratory list and on the plastic boxes with the faecal samples in it. This way samples can be quickly found when needed. Until examination the faeces is stored in a refrigerator at 5°C for a maximum of 4 days.

Online questionnaire

When the faecal samples arrive at the lab, it is registered in the system with the date of receiving. Participants receive an e-mail with the message that their package has been arrived and they have to fill in a questionnaire. The first questionnaire contains questions about possible risk factors that could be association with a patent infection. Questions were asked, for example, about the environment the dog lives in, demographical data, food, health status of the dog etc. The following faecal sample that are send in, participants are asked to fill in a questionnaire about changes in the just named subjects. All these answers are collected and linked to the unique participants number.

Faecal examination

Usually the faecal samples will be processed the day they arrive at the laboratory. Only when there are too many samples to process in one day, they will be kept in the refrigerator to be processed the next day. We work together with the VMDC (Veterinary Microbiological Diagnostic Centre).

Faecal samples are initially pooled, which means that faeces of two dogs will be examined together. This way a larger number of samples can be processed in a day. Only samples of dogs that have been positive for *T.*canis earlier, were dewormed or the amount of faeces is less than 6 grams (otherwise there is not enough faeces to examine for a second time at the VMDC) are processed individually. To look for eggs of *Toxocara canis* the centrifuge- sedimentation-flotation method (CSF method) with sucrose solution (specific gravity of 1.270-1,290 kg/dm3)29 is used. 3 – 5 grams of faeces per dog is weighed on a scale and the faeces itself is macroscopically examined for parasites and consistency, which will be noted in the laboratory list. A clean spatula is used to transfer the faeces from the bag it has been send in, to a piece of plastic on the scale. The faeces will be put in a mortar together with 55 mL tap water in case of a single faecal sample, and 110 mL tap water in case of a pooled faecal sample. The faeces will be crushed and blended with the water until the faeces is well suspended and eventually poured into a sieve on top of a tube, closed at the bottom, to collect the water and suspended faeces. The larger parts stay in the sieve and the smaller parts, including the worm eggs, are collected in the tube. In the sieve larger worms, bones or other dietary elements can be seen. The collected water with possibly worm eggs will be poured into a centrifuge tube, after swerving. Those tubes will be put in a centrifuge where the particles will end on the bottom of the centrifuge tube and the supernatant will be poured off. Subsequently the tube will be filled for one third with sucrose solution and will be mixed with a vortex mixer. The centrifuge tube will be filled up to the top with sucrose solution till a small meniscus on which a coverslip can be placed. After centrifugation the eggs will be swingled on the coverslip which will be removed and placed on a glass slide. After usage materials are kept in hot water of 80°C for a while to inactivate the embryonated eggs, and are washed with a detergent. Coverslips and glass slides are thrown away after microscopic examination.

The product of the centrifuge sedimentation flotation technique can be thoroughly examined under the microscope with 10x10 magnification. The preparation is systematically screened for *Toxocara canis* eggs. The eggs are 75-80 um in size, have a dark, round, single-celled embryo in a thick shell wall that has got pitts.30 Besides *Toxocara* eggs, there will also be screened for *Strongyl type* eggs

like *Uncinaria*, *Trichuris* sp., *Capillaria* sp., *Taenia* sp., *Isospora* spp. and *Giardia cysts.*

When a *Toxocara* egg is found, this will be noted on the laboratory list that keeps the record of all the samples processed during the day. The pooled monsters that are found positive for *T.*canis eggs are coproscopically examined by the VMDC and the results will be written on a form.

Fig. 1 - *Toxocara* egg.30

These forms eventually will be filled in into the system so all the results can easily be found.

Processing of the results

The results of the microscopic examination are processed and collected in a Microsoft Excel file. The results are also communicated to the owners of the dog(s) via the e-mail after participants have filled in the questionnaire. When the result of a pooled or a single sample is negative for *Toxocara*, the owners will receive a mail that says no eggs are found in the faeces of their dog(s) and they can send in a new sample next month. When the result of a pooled sample is positive for *Toxocara,* the samples will be examined individually by the VMDC using zincsulphate (specific gravity of 1,34 kg/dm3).29 When the result of this single sample or a single sample examined by the team is positive, the owners of the dog(s) are informed and asked to send in another sample. For this sample the dog has to be on a leash for three consecutive days to prevent it from eating faeces, because eggs can also pass the gastro-intestinal tract after eating contaminated faeces. After these three days faeces has to be collected and send in again. These samples are examined as an individual single sample and a negative result means that the dog probably has eaten faeces with *Toxocara* eggs last time. A positive result could mean that the dog has a patent infection with *Toxocara.* Owners are informed about the result and Drontal is send to deworm the dog when found positive for the second time. Two weeks after deworming, owners are asked to send another faecal sample to check if the dewormer has worked. These samples will also be examined separately. A dog could be patent infected when there are at least two times *Toxocara* eggs found in the faeces.

Supplementary online questionnaire

Because the goal of this research is to look for a relationship between dogs eating raw meat and a patent *Toxocara* infection there are some additional questions necessary about the origin of the meat that is being fed. That is why a supplementary online questionnaire is send to owners feeding their dog(s) raw meat. Questions that are asked deal with the kind of meat the dog eats (fresh or frozen meat), from which species the meat comes from and how these animals are kept, if the dog eats animals of prey, how the meat is preserved and the same questions are asked about snacks. This way more information is gathered about the origin of raw meat and it will be possible to get an indication about the possible risks of the meat*.*

Statistical analysis

We evaluated the association between dogs eating raw meat and a patent *T.canis* infection. We also looked for an association between a patent infection and fresh versus frozen meat, the species origin of the meat, the housing of these species, eating prey animals, the place where people bought the meat, the brand names of commercially made frozen meat, the way the meat is preserved and for the time the meat has been preserved. The values are binary, therefore a logistic regression is used. A P-value of <0,05 was considered significant. SPSS is used to analyze all data.

**Results**

There were 905 participating dogs in this research we received at least one time a faecal sample from. We statistically analysed all factors of risk for a patent *T.canis* infection associated with raw meat. All the others possible risk factors were not included in this study.

Overall 88,7% of all these dogs had no patent *T.canis* infection. We call a dog patently infected when *T.canis* eggs are found in a single sample and the *T.canis* eggs are found again after three days not eating faeces. 11,3% of the 905 participating dogs were found positive for a patent *T.canis* infection. 68,2% of the 905 participating dogs ate raw meat and 31,8% did not eat raw meat. In 8,6% of the raw meat eating dogs, we found a patent infection. For dogs that did not eat raw meat 17,1% were patently infected with *T.canis.* We found a significant association (P<0,001) between dogs not eating raw meat and a patent *T.canis* infection.

Fig. 2 – Number of dogs that do not eat raw meat and dogs that do eat raw meat. In green the number of dogs in these separate groups that have a patent *T.canis* infection.

In the additional questionnaire we asked owners of

dogs who fed their dog(s) raw meat about the origin of the meat and the housing system these meat producing animals were kept in. From the group of dogs that ate raw meat 20,8% ate meat from free-ranging animals, 16,8% ate meat from intensive livestock farming and 23,1% ate meat from the wild obtained by hunting. These dogs did not solely fit into one group, most dogs were fed meat from more than one origin. From 64,4% of the raw meat eating dogs the origin of the meat was unknown. No association was found between a patent *T.canis* infection and intensively livestock farmed animals (P=0,780) and wild animals (P=0,648). However, an association was found between a patent infection and animals that are fed not free-ranging animals (P=0,017). 4,3% of the dogs that eat raw meat from free-ranging animals have a patent *T.canis* infection and 14,5% of the dogs that do not eat raw meat from free-ranging animals have a patent infection.

21,3%

47,1%

31,5%

Fig. 3 – The division of dogs at risk for a patent *T.canis* infection, dogs not at risk and the percentage of dogs of which the origin of the meat they eat is unknown.

We also subdivided the group, consisting of 905 dogs, into a group at risk for a patent *T.canis* infection according to the chance of infection of the meat producing animal, and a group not at risk for a patent *T.canis* infection. The group at risk consisted of dogs that ate raw meat from free-ranging animals or wild animals from hunting. The group not at risk consisted of dogs eating dry or canned dog food and dogs eating raw meat that comes from animals that are kept inside with a low risk of getting infected by *T.canis*. 8,3% of the dogs from the group at risk are patently infected and

31,5%

21,3%

47,1%

16,1% of the dogs from the group not at risk are patently infected. Again we found a significant association (P=0,014) between the group not at risk and a patent *T.canis* infection.



Although no association was found, we still looked at the other possible factors of risk concerning the raw meat. There was no significant association found between frozen meat (P=0,326 ) or fresh meat (P=0,878) and a patent infection. Nor between the animal species where the eaten meat originated from and a patent infection: chicken (P=0,924), turkey (P=0,197), cow (P=0,455), pig (0,585), sheep (P=0,255), goat (P=0,937), horse (P= 0,166), birds (P=0,278), wild animals (P=0,366) and exotic/special animals like lamas (P=0,709).

Between eating animals of prey and a patent *T.canis* infection, no significant association was found (P=0,958).

The place people bought their meat

Fig. 4 – Number of dogs that are not at risk for a patent *T.canis* infection and dogs that are at risk for a patent infection. In green the number of dogs in these separate groups that actually have a patent *T.canis* infection.

 no significant association was found

 between the biological butcher

 (P=0,476), the regular butcher

 (P=0,077), the supermarket (P=0,842), the pet shop (P=0,175), the internet (P=0,909) and the hunter (P=0,660). Only meat bought at a special address showed a significant association (P=0,041) between buying the meat from a special address and a patent *T.canis* infection. 13,6% of the dogs that ate meat bought from a special address showed a patent *T.canis* infection and 6,6% of the dogs that did not eat meat bought from a special address showed a patent infection.

We also compared different commercial available raw meat brand names (32 in total) all with P-values above 0,05, except for ‘Smuldier’, so no significant association was found. ‘Smuldier’, however, showed a significant association (P=0,010) between not eating ‘Smuldier’ and a patent *T.canis* infection. 4,6% of the dogs that ate ‘Smuldier’ were patently infected and dogs that did not eat ‘Smuldier’ were found 11,4% patently infected.

Between the different ways to keep the meat and a patent infection, no significant association was found: freezer (P=0,751), refrigerator (P=0,667) and room temperature (P=0,477). Also between the period the meat was preserved and a patent infection no association was found: several days (P=0,736), a week (=0,826), several weeks (P=0,782) and several months (P=0,839).

**Discussion**

In this independent research from the Faculty of Veterinary Science of the University of Utrecht, the association between dogs eating raw meat and a patent *T.canis* infection was investigated. A representative number of 905 dogs participated by owners sending in faeces every month and filling in a questionnaire. This group consists of dogs of which the owners have send in at least one faecal sample during the research project or had filled in the questionnaire. Because zero, one or two faecal samples are not representative for this research to find patent infections, some dogs should have been excluded. Thereby, we might have attracted a specific group of people for this research that might not be representative for the dog-owners in the Netherlands. People participating care a lot for their dog(s) and are willing to do more for their dog(s) than just the regular caring.

We found a patent *T.canis* infection in 11,3% of these dogs which means that there are *T.canis* eggs found in at least one monthly faeces sample and the repeated faecal sample after three days not eating faeces. This last sample is asked to send in to distinguish a patent infection from passaging eggs. Eggs found in the faeces can originate from a worm present in the intestine of the dog. But these eggs can also be eaten and leave the dog with the faeces so there is only a passage of these eggs.4 When a second sample, after three days on the leash to prevent the dog from eating faeces from the environment, is still positive for *T.canis* eggs, it is most likely that this dog has a patent infection. However, we do not know whether the dog really does not have eaten any faeces that can contaminate the faecal sample of the dog. Thereby it is possible that the faecal samples are contaminated during the process in the lab, although all materials are thoroughly flushed with water. Repeated samples are asked when one sample is found positive. This way we can check if the dog has not been eating faeces from other dogs with *T.canis* eggs or we have contaminated the sample during the process.

88,7% of the dogs showed no patent infection of *T.canis.* Unfortunately not all owners send in faeces every month. Besides, when a dog is found positive for *T.canis* in the second sample as just described, deworming pills (Drontal) will be send to the owner. This because *T.canis* is a potential zoonotic risk and especially children can develop clinical signs.5, 23 These two aspects result in loss of information about the course *T.canis* takes in one single dog.

Another factor that makes this research not continuous is the fact that the people working on it were variable. There are two continuous people in the process. The work in the laboratory will most of the time be done by students who stay at the project for 3-5 months and who are no experts, especially in the beginning. Because of the good distinguishability of *T.canis* eggs, recognizing these eggs is learned quickly. Besides, samples will be checked in the beginning to prevent eggs being missed in the sample.

There is a period of time after sending it in we do not know what happens with the samples and it is unknown how old the samples are when we receive them. It is possible that owners do not send in the faeces immediately after collecting it. The faeces has to be send via the mail and we do not know exactly the circumstances the packages are kept during this period of time and how they will be stored. *T.canis* will remain viable for a long period and under variable circumstances. However, besides *T.canis* we also look for other parasites like *Giardia, Cysto-Isospora, Trichuris vulpis, Taenia* sp. and *Strongyl* typeeggs. Especially *Giardia* is a fragile parasite so faecal samples have to be looked at as soon as possible.29 In the laboratory we process the samples the day they arrive or we keep them at 5°C for a maximum of 4 days.

In our laboratory we use the Centrifuge Sedimentation Flotation (CSF) technique and a sucrose solution as flotation medium with a specific gravity of 1,27-1,29 kg/dm3. This flotation medium is used for detection of *T.canis*, *Uncinaria* sp., *Trichuris* sp., *Capillaria* sp. and *Isospora* sp. 5 The specific gravity of *T.canis* is 1,0900 kg/dm3 so these eggs will float in this solution.5, 29. When we find parasites and oocysts like *T.canis*, *Giardia*, *Isospora* sp. and *Trichuris* sp. in pooled samples, we send both faecal samples to the VMDC. Here they use zincsulphate as flotation medium with a specific gravity of 1,34 kg/dm3. This medium will find *T.canis* eggs too, so to look for *T.canis* the difference in flotation medium should not be a problem. And *Giardia* cysts are better maintained in zincsulphate solution.5, 29

One single female *T.canis* worm produces 200.000 eggs a day.1, 2 So when there is one single worm producing 200.000 eggs dispersed in the faeces in one day, there should be enough eggs to be found. Thereby, centrifugation leads to a significant higher egg count of *T.canis* compared to simple flotation.29 So the centrifuge technique should be used for detecting *T.canis* eggs. Sedimentation helps to reduce the interference of substances with a very low specific gravity (lower than parasite eggs and oöcysts) in the faeces. The supernatant with these substances will be poured off, this way they will be eliminated.31

A large number of dogs, 68,2%, ate raw meat in our study. In this group all dogs are included that would eat any amount of raw meat, so dogs eating dry food with once a week some raw meat are also included. In the Netherlands 15% of the dogs eat a diet of just raw meat (Personal communication Drs. R.J. Corbee). This group is bigger in our study, probably because we defined the group of dogs that eat raw meat in a different way. But also the way information was distributed about this research could be taken into account. Especially fora on the internet reached a lot of people feeding their dog(s) raw meat.

There are many factors of risk that influence the occurrence of a patent *T.canis* infection. Preparation of meat with temperatures of 70-78°C will inactivate *T.canis* eggs and larvae. That is why eating raw meat is a potential factor of risk for developing a patent *T.canis* infection.21 The statistical analysis showed that there is a significant association (P<0,001) between dogs not eating raw meat and a patent *T.canis* infection. This means that dogs that do not eat raw meat, so dogs eating dry or canned food, are more often positive for a patent *T.canis* infection. This is not in line with our expectations*.* It is possible that meat producing animals do not have the chance to get infected with *T.canis* because no dogs are allowed and good fencing. However, wild animal do have the change to get infected because they live in the same area dogs and wild foxes live and here no association was found either. Furthermore, there are articles available where *T.*canis larvae are found in tissue of meat producing animals.11, 14, 33

It could be possible that encapsulated *T.canis* larvae are not infective anymore by the time they get eaten by a dog. However, production animals are slaughtered when the meat profit is highest and these animals shall not become very old. *T.canis* larvae survive in chickens for 3,5 years 10 and they are slaughtered at 42 days of age.34 In sheep larvae survive at least 7 months33 and sheep are slaughtered from 6 months of age on. 35 Larvae in pigs survive more than a month 10 and pigs are slaughtered at 6 months of age.34 When these animals have the possibility to go outside, they are constantly reinfected with *T.canis* and will be infected by the time they have to get slaughtered. Thereby, a study of Sasmal et al. found *T.canis* larvae in mice fed liver of an infected piglet. Although it was a small number of larvae found in the mice, this shows that *T.canis* is capable of adapting and migrating in a second paratenic host. 13 No research is done into the consequences of dogs eating encapsulated larvae in meat and how viable these larvae are in the dog. But this explanation will probably not apply to the results found.

From 2 to 3 months of age on, dogs develop immunity against *T.canis.* The migratory pattern of the larvae changes from mostly tracheal migration to mostly somatic migration.1, 2, 4, 6 This immunity consists of a delayed hypersensitivity reaction in the lungs against the L3 larvae which mostly prevents the larvae from migrating through the lungs. 4, 6 In the intestine immunity develops specific for L3 stage larvae. When these larvae penetrate the intestinal wall, an allergic reaction prevents them from further penetration. 4, 6 Stages other than L3 can develop free in the intestine into adult worms.4 However, it is unclear how it is possible that there is somatic migration in adult dogs even though there is also gastro-intestinal immunity against L3 stage larvae. More research has to be done on this subject.

Thereby, other risks for an infection with *T.canis* can be the geographical location, environmental factors, behaviour of the dog, health status of the dog and habits of dog and owner etc. Further research on these risk factors is necessary.

Furthermore, we subdivided the participating dogs in a group at risk for a patent *T.canis* infection and a group not at risk for a patent *T.canis* infection. The group of dogs at risk are dogs eating meat from free-ranging and wild animals that could be infected. The group of dogs not at risk are dogs eating meat from animals that are kept inside and dry or canned dog food. A lot of people, 64,4%, did not know what the origin of the meat was, this makes the outcome less reliable. The statistical analysis in this group also showed a significant association (P=0,014) between the group not at risk and a patent *T.canis* infection. This means that dogs in the group that are not at risk are more often *T.canis* positive in their faecal samples. The same reasons as mentioned above could be an explanation.

We looked for risk factors within the group of dogs that eat raw meat. Because no questions were asked about, for example, the origin of the meat in the questionnaire send to owners, a supplementary questionnaire was send to owners who feed their dog(s) raw meat. Some questions appeared not to be clear to owners. For example, we asked if their dog(s) eat(s) animals of prey. We meant to ask about wild animals like mice, rabbits, birds, that are caught by their dog and may be eaten. However, people that feed raw meat, sometimes give their dog(s) dead animals of prey so this questions was not interpreted the way we intended.

Also the questions about the way people preserve the meat and for what period were misinterpreted. We wanted to know whether meat was kept in the freezer, refrigerator or at room temperature and for what period of time so we could get an indication of the chance of survival for the encapsulated larvae. However, people preserving the meat in the freezer answered most of the time with all these possibilities because they keep the meat in the freezer for a while, defrost it on room temperature and then kept it in the refrigerator. So these results may not be reliable and better formulated questions should have been asked.

Although no association was found between eating raw meat and a patent *T.canis* infection, we also looked at different factors within the group of raw meat eating dogs. We used a logistic regression as statistical analysis. However, more factors are important so better a multi-factorial analysis should have been made.

We expected the free-ranging animals to be at higher risk of passing a patent infection.14 However, a significant association (P=0,017) was found between dogs that did eat meat from not free ranging animals and a patent *T.canis* infection. No proper explanation can be found for this result. In the additional questionnaire send to people who feed their dog(s) raw meat, 64,4% did not know the origin of the meat and the way these meat producing animals are kept. So it is hard to conclude anything from these data. No association was found between a patent infection and intensively farmed meat producing animals or wild animals. No association was found either for the animal species where the meat originated from. Also no difference was found for eating animals of prey although we assumed the risk should be higher because wild animals have the possibility to get infected with infective *T.canis* eggs from soil.

No difference was found between frozen meat and fresh meat. A study of O’Larcain et al. in 1995 showed that after 34 days of freezing in temperatures ranging from -16,37°C up to

-9,15°C, 22,84% of the *T.canis*  larvae were still viable. 36 A more recent study showed that larvae were not motile and not infective after 24-48 hours in -25°C.22. However, most freezers are -18°C. According to this information, possibly not all the larvae are killed by freezing in an ordinary freezer. Unfortunately no information is available about the time a larvae can be viable in -18°C.

For the place people bought their meat, no significant association was found between the shop and a patent *T.canis* infection. Only dogs that ate meat bought at a special address were significant associated (P=0,041) with a patent *T.canis* infection. It was not specified what a special address could be so the origin of the meat is unknown. It is hard to tell what the factor of risk is in this group of dogs that eat meat bought at a special address.

We also compared different commercial available raw meat brand names (32 in total), all with P-values above 0,05 so no significant association was found with the exception of ‘Smuldier. A significant association was found (P=0,010) between not eating ‘Smuldier’ and a patent *T.canis* infection. This should mean that dogs that do not eat ‘Smuldier’, more often have a patent infection. However, a lot of people feeding frozen raw meat to their dog(s) feed ‘Smuldier’ (40,2%). The percentages of dogs fed with other brand names of commercially available frozen meat varies between 0,3% - 10,4%. Because of the very large number of dogs that eat ‘Smuldier’ against the very small number of dogs eating other brand names, it cannot be said that other brand names are more prone to give a patent infection.

For the different ways to preserve the meat, no significant association was found. Also for the period the meat was preserved no association was found. These results are not reliable because many people misinterpreted the questions.

Conclusion

A patent infection with *T.canis* is a problem with many different factors of risk. In this study we focused on the risk of feeding raw meat to dogs. The results show that raw meat does not influence the chance of getting a patent *T.canis* infection although this is not in accordance with our expectations. More research should be done on the dogs gastro-intestinal immunity, the viability of L3 larvae in paratenic hosts and the possibility of infecting dogs. Thereby other factors can be more important for the development of a patent infection. So further research has to be done to investigate these other factors of risk for a patent infection. Still there is a high percentage of foxes, 44,3%, with a patent *T.canis* infection. 27, 28 These two *Canidae* should have something in common that will lead to a patent *T.canis* infection.

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Information on the research of *Toxocara canis* is also available at [www.parasietenwijzer.nl](http://www.parasietenwijzer.nl) .

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