# **Correlation between the fecundity of *T. canis*, the worm burden and sex distribution in Dutch red foxes (*Vulpes vulpes*) with a comparison of McMaster and mini-FLOTAC.**

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## Abstract

**Background:** *Toxocara canis* prevails to be a prevalent zoonotic agent in the environment. The natural sources of its eggs, such as the red fox (*Vulpes vulpes*), have to be more deeply investigated. There is still a lack of understanding of their role in the zoonotic transmission of infective stages of *T. canis* in relation to the public health. We do have a much deeper understanding of the role of household dogs (*Canis lupus familiaris*) on the contamination of the environment with infective stages of *T. canis* than we do on foxes.
**Objective:** In this paper the main focus lies on possible relations between the age, gender, and body condition score (BCS) of the host with the intestinal worm count, the gender of the worms, the fecundity of female worms as well as the eggs per gram faeces (EPG).
**Method**: The data used in this study has been derived from dissections of foxes, qualitative as well as quantitative coproscopical examinations and counting as well as sexing of the worms. The quantitative EPG counts were done with two different techniques, the proven McMaster and the new mini-FLOTAC method.
**Results**: Only the BCS correlated positively with the intestinal worm count. Neither the age nor the gender of the foxes could be associated with the EPG count or the intestinal worm count. The compared quantitative techniques had a high concordance and verified earlier results from the literature.
**Conclusion**: The mini-FLOTAC technique has proven to be a viable but more time intensive alternative to the McMaster technique, with a higher resolution and a lower detection threshold.

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## Introduction

The nematode *Toxocara canis* has a worldwide distribution and its definitive hosts are canids like dogs and foxes. This roundworm has a direct life cycle in which prey animals, but probably all birds and mammals may function as paratenic hosts. These paratenic hosts can get infected when they ingest embryonated eggs from the environment. When animals, including humans and canids, ingest embryonated eggs, larvae will start a migration through the body and damage encountered tissues. In potential paratenic hosts this migrations ends when the larvae become dormant somatic tissue larvae. In canids younger than two months of age the migration takes a specific route necessary for the reproduction of *T. canis,* a hepato-tracheal migration (HTM). Gradually older canids develop an age-resistance and migrating larvae also become somatic tissue larvae. These migrations can lead to symptoms varying in severity in different types of hosts.
Canids get infected very early in life and during this period they experience the most severe symptoms and shed the highest number of eggs in their lifetime. Symptoms in canid neonates include pneumonia from the HTM and a distended abdomen (pot belly) from the worm burden and the resulting dysbacteriosis. With progressing age from about two months onwards, canids develop a resistance, the HTM gets inhibited and fewer symptoms become apparent. The aforementioned resistance leads to somatic tissue larvae instead of a HTM from newly ingested infective eggs. However, they can develop new patent infections directly in the intestines without HTM by ingesting paratenic hosts (Overgaauw 1997).
Humans, especially children, get infected most often due to the ingestion of soil containing infective eggs of *T. canis*. The disease patterns in humans are also a result of migration of larvae and are consequentially called visceral larva migrans (VLM), that can lead to inflammation of all kinds of invaded tissues and occular larva migrans (OLM), which describes the formation of granulomas behind the retina of the eye and can lead to blindness (Despommier 2003).

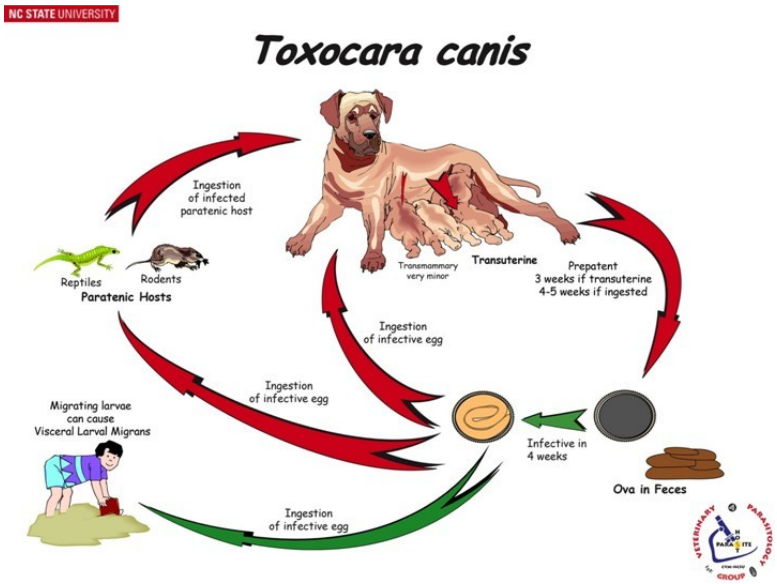
Source of infective eggs is soil in the environment, which became contaminated by faeces derived from canids and felids with a patent infection of *Toxocara* spp.. These eggs endure a wide range of environmental conditions and stay infective for at least a year in optimal conditions.
The zoonotic aspect of *T. canis* makes the presence of its infective eggs in the environment a public health issue. As a consequence studies by Morgan et al. (2013)as well as Nijsse et al. (2015) were done to evaluate the different influxes of *Toxocara* spp. eggs into the environment. One of these influxes of *T. canis* eggs is facilitated by foxes and the amount of knowledge over their contribution is one of the lowest. Even though there are much fewer foxes in the Netherlands than dogs, the prevalence of *T. canis* in foxes is much higher with an average between 33.3% and 50.0%, depending on the area they live in, compared to an average between 1.8% and 8.4% in dogs. This low but steady contribution of foxes form a continuous natural reservoir for infective eggs of *Toxocara canis* in the environment. Morgan et al. (2013) shared that “the extent to which defecation by foxes of different ages contaminates environments shared by humans is a complex question, and not one we are equipped to address here”.
This paper does not try to solve this complex question but aims to add to the knowledge about *T. canis* in Dutch red foxes, so future research can be built on already existing data.
What this paper does try to achieve is to look whether there are associations between characteristics of the worms, including the fecundity, the gender distribution ratio, the total worm count and data derived from the foxes including age, gender of the foxes and the body condition score (BCS).

## Life Cycle

*Toxocara canis* has four modes of transmission: transplacental, lactogenic, direct via ingestion of embryonated eggs and via ingestion of paratenic hosts containing somatic tissue larvae.
After an embryonated *T. canis* egg is ingested by a fox it reaches the intestines where the third stage larva hatches and starts its migration. In fox puppys up to two monts the larva migrates via the liver and the vascular system through the lungs into the trachea where it makes its way up the airway (HTM). After being coughed up and swallowed again, the larva starts to develop into a productive adult worm in the small intestines.
In foxes older than two months of age the migration starts the same way, but due to acquired immunity (age-resistance), the larvae make a somatic migration and become dormant as somatic tissue larvae in the invaded tissues. When non canids ingest infective eggs the hatching larvae also undergo a similar somatic migration and lay dormant as an infective stage in the invaded tissue. After consumption of a paratenic host by a canid, in most cases there is no migration. Instead the development into an adult worm starts directly in the intestinal lumen, leading to a patent infection. This direct development appears only when the larvae had a chance to develop far enough in the paratenic host. If the paratenic host has ingested the infective eggs very recently, the ingestion of the paratenic host by a canid still results in a migration. When the larvae in the paratenic host have migrated, got the chance to develop further and became dormant somatic tissue larvae, the ingestion of the paratenic host leads to a direct development in the intestines of the canid without a migration through tissues.
The prepatent periods in canids are similar. Foxes show a prepatent period with infective eggs of 33 to 41 days post infection (dpi), while the range in household dogs is 30 to 34 dpi. This time span is shortened to 21 dpi in foxes if developed larvae were derived from paratenic hosts and are then able develop directly in the intestines without a HTM (Saeed, Taira, and Kapel 2005).

During pregnancy, elevated levels of prolactin shift the immune response of the vixen (female fox) towards a Th2 response, thus an elevated immunoglobulin production and changed immune system regulation. As a consequence the somatic larvae start to migrate again. These larvae can pass the placenta and infect the pups intrauterine via the umbilical cord. After the pups have been born they can also get infected orally via the milk as the larvae migrate through the mammary glands. (Overgaauw 1997; Saeed and Kapel 2006; Saeed et al. 2005)

Figure 1: Life Cycle of T. canis (NC State University)



## Epidemiology

Foxes as well as household dogs are able to develop patent *T. canis* infections. They develop infections congruently as described earlier. Both species acquire an age resistance within their first year of life and both shed much fewer eggs and less frequent when they are adult.
Here is where the similarities start to end. Unlike household dogs, foxes roam freely in their habitats. The feeding pattern of foxes is mostly dependent on the area they live in and what animals, plants and carrion are available to serve them as a food source, which is also dependent on the time of year. Prey animals are potential paratenic hosts and can be the main source of patent infections in foxes older than six months.
Due to the tendency of adult foxes to be solitary except for the time around the mating season, another way to be exposed to infective eggs is contact with contaminated soil while foraging for earthworms and insects. Moreover, the sticky nature of *T. canis* eggs leads to contaminated fur which becomes a source of infection during grooming. Foxes have not been observed to indulge in coprophagic behaviour, in contrast to dogs. Therefore coprophagy as a source of shedding eggs for foxes appears to be highly unlikely. Dogs do indulge in the consumption of faecal matter of all kinds, including that of cats and foxes. However, potentially contained eggs in the consumed faeces didn't have the time to sporulate and become infective as long as the faeces is still consumable and not already diluted by erosion.
The most likely way for recurring patent infections in household dogs is either because of alterations of the endocrine system, the immune system due to stress and pathologies or the consumption of low levels of infective stages. Infective eggs may be contained in remnants of former paratenic hosts or larvae contained in fresh meat(Fahrion, Staebler, and Deplazes 2008). Some of the most important risk factors for shedding eggs in household dogs include being younger than one year, being older than seven years of age, being kenneled within the last two months and to get fed a commercial diet. The degree of freedom allowed to household dogs is nearly linearly correlated to the risk of shedding eggs and poses the biggest risk factor. Being a farm dog is also a risk factor, but on the level of no to little unsupervised time, while nearly complete freedom of household dogs increases the chance more than eleven fold. The contamination of soil in rural areas can be on the same level due to the shedding of eggs by foxes, as well as due to the low faeces removal rates from dog faeces in rural areas. In urban environments, the faeces removal rate is higher, but parks in cities are hotspots for infective stages of many parasites (Ferreira et al. 2017; Nijsse et al. 2014) .

The most *T. canis* eggs shed into the environment from single individuals comes from lactating vixens and bitches as well as their offspring. The lack of age resistance in the cups often leads to patent infections and as a consequence to an increased output of eggs. All unembryonated eggs, ingested by the mother through licking the excretions of their cubs, will pass the mother and add to the excreted eggs into the environment.
While lactating vixens roam freely and have to eat enough to be able to care for their cubs, nursing bitches are kept closer at the side of their owners and their faeces as well as the faeces of their cubs is more readily discarded. Beside the removal of faeces, there is also an anthelmintic therapy advised for household dog pups and their mothers, which if applied, reduces the egg output from mother and litter.
However, juveniles are not the biggest contributors in the general picture. The vast majority of *Toxocara* spp. eggs in the Netherlands is excreted by adult dogs if the average of all important contributors (household dogs, household cats, stray cats and foxes) is taken. This is due to the much greater numbers of adult dogs compared to rather small number of dog pups. Nonetheless, there are quite big differences between urban, intermediate and rural areas. In urban areas stray cats are the biggest contributors with 80.7% of all shed eggs, while foxes only add less than 0.01%. In intermediate areas dogs are ahead with 54.8%, in contrast to rural areas where foxes shed the most, with 41.3% contribution of shed eggs into the environment (Nijsse et al. 2015).

# Materials & Methods

## Retrieval of worms and faeces

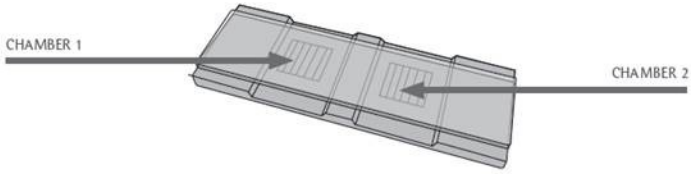
For this study, 78 foxes from the North Eastern part of the Netherlands that were shot by local hunters were sent to the RIVM Bilthoven (National Institute for Public Health and the Environment). After delivery the foxes were frozen at -80°C for a week. This measure was taken to kill off all *Echinococcus multilocularis* eggs possibly present in or on the foxes. When this mandatory process had been completed the foxes were sent to the Department of Pathology of the Faculty of Veterinary Medicine at Utrecht University, where they were thawed, weighed and measured from nose to anus. The approximate age of the foxes was estimated on the basis of the external appearance of the cuspids and classified as animals younger than one year or older than one year. Moreover, the gender was determined and special traits of the individual fox were noted.

During the dissection,the faecal content of the colon and rectum was collected, if present. The small intestines, from the pylorus to the ileum, were ligated and sent back to the RIVM for a different study on *E. multilocularis*, but also for retrieval of *T. canis* worms present in the intestinal lumen. The collected worms were sent to the Department of Infection and Immunity (I&I) of the Faculty of Veterinary Medicine of the Utrecht University to be counted and to determine the sex. Other samples were taken for different studies on different parasites.

## CSF and EPG via McMaster and mini-FLOTAC

The first step in determining whether eggs were present in the faeces was to perform a centrifugal sedimentation flotation technique (CSF). Three grams of faeces were put into a pestle and were suspended in enough water, so the resulting suspension was able to fit comfortably in a test tube, depending on the consistency of the faeces. This suspension was sieved through a tea strainer into a small glass container to extrude large particles present in the faeces. A test tube filled with this suspension was centrifuged at 3000 rpm for two minutes with a Hettich Rotofix 32 that has a radius of 135mm and reaches a g-force of 1358.2 g. The supernatant was decanted and an amount of sucrose solution with a specific gravity of 1,27-1,30 g/cm$^3$ was added to the remaining sediment. With a vortex-mixer, the sediment and the sucrose solution were mixed until a homogeneous suspension was formed. More sucrose solution was added to the test tube until a slight positive meniscus formed at the top. With a coverslip added to the top of the tube it was centrifuged again two minutes at 1358.2 g. The coverslip was then taken perpendicularly off the tube and put on a slide. This slide got inspected systematically under a microscope at 100x and 400x magnification and all eggs/oocysts of the different helminth and protozoan species were noted.
If *Toxocara canis* or *Capillaria* spp eggs were present, the next step was to do a quantitative examination of the faeces with the McMaster technique and the relatively new mini-FLOTAC technique. For this procedure the same sucrose solution was used with the same specific gravity of 1,27 – 1,30 g/cm$^3$. Three grams of faeces were put into a pestle and got slowly mixed with 42 ml of the sugar solution while being suspended with a mortar. The suspension got cleared of the bigger parts of debris by filtering it with a tea strainer and squeezing it with a pestle into a small glass container. This suspension was poured into a falcon tube which served as a reservoir for the chambers to be filled with a pipette. Just before each fill process the tube was slowly but thoroughly slewed to homogeneously resuspend the debris, containing eggs, without introducing air bubbles.

Figure 2: McMaster slide



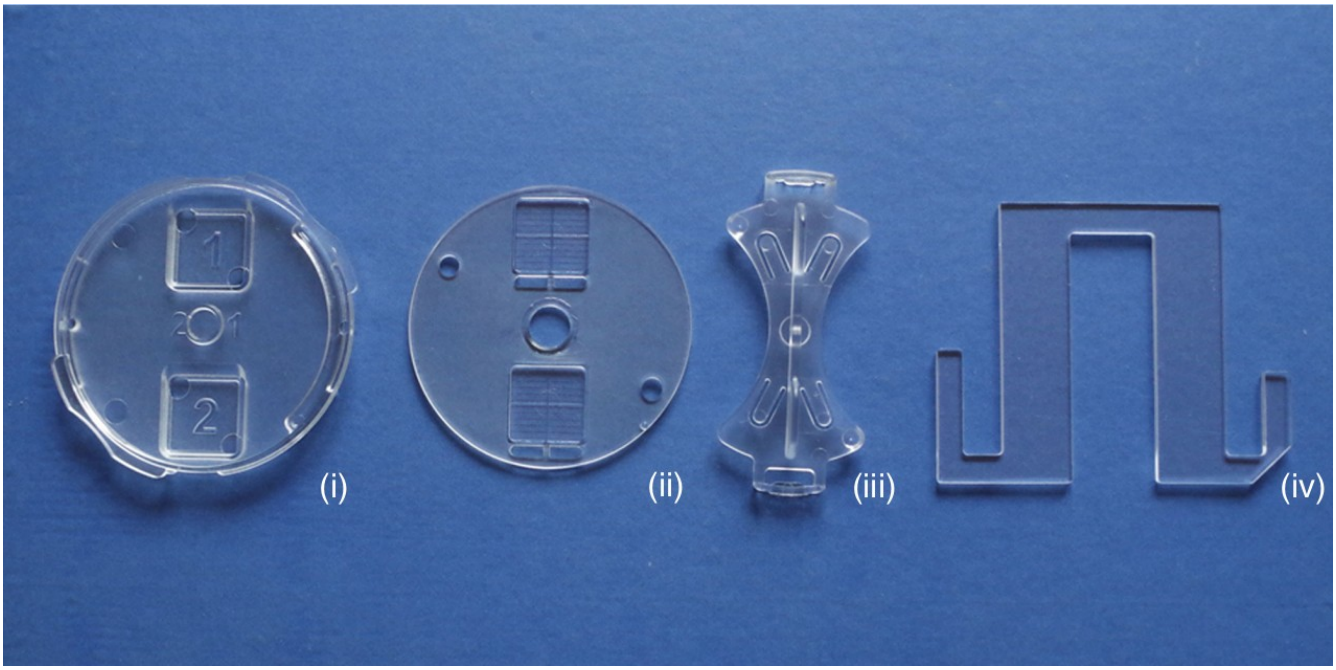

$$\text{Eggs / g of Faeces (EPG)} = \frac{(\text{Chamber A} + \text{Chamber B}) \times 100}{2}$$

The McMaster technique consists of a microscope slide made of two glass sheets that form two counting chambers. These chambers are filled with the suspension for examination and after the minimum waiting period of seven minutes the contained eggs are then counted with 100x magnification.

With thanks to woodleyequipment.com

The mini-FLOTAC device consists of two polymer discs that fit together to form a single disc, but the two parts can be turned against each other by 90°. One end of the 90° turn comprises the open, the other end the closed position.

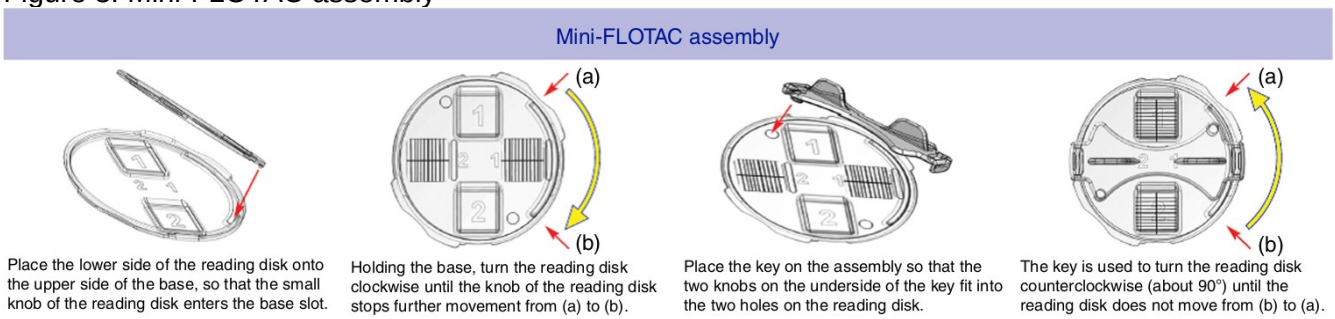
Figure 2: Parts of the mini-FLOTAC device




(i) lower disc with chambers, (ii) upper disc with grid, (iii) locking key, (iv) microscope holder. With thanks to Cringoli et al. 2017

In the closed position the device is held at an angle of 45° with the filling slits in the higher position. The resuspension, that has been slewed up till this moment is taken out of the tube with a pipette and is filled into the chambers until a slight positive meniscus is formed on top of the two slits. It might be necessary to add a few more drops of the suspension after a few minutes to regain the menisci and ease the turning of the key and to prevent the formation of bubbles. After the minimum waiting period of seven minutes, the key is turned 90° counter clockwise into the open position and the key is removed. With this motion, the most upper layer of the suspension is sheared off and is dragged with the counting raster into the open position. The device is put into a plastic holder for the microscope and the counting raster can be used to count the eggs with 100x magnification.

Figure 3: Mini-FLOTAC assembly




Cringoli et al. 2017

The outcomes of each counting technique have to be multiplied with their corresponding factors. The used suspension of 42ml sucrose solution and 3g faeces results in a ratio of 1:15. Each grid of a McMaster slide equals 0,15ml with 6 rows in a grid, this equals 0,3ml of suspension for both chambers. Considering the thinning ratio of 15 divided by the volume of 0,3ml, one counted egg equals 50 EPG. This calculation also has to be performed with the mini-FLOTAC device which contains 1ml per chamber with 12 rows per grid. The thinning ratio of 15 divided by the volume of 2ml equals 7,5. Each counted egg with the McMaster technique equals 50 EPG, while with the mini-FLOTAC each egg stands for 7,5 EPG.

### Fecundity

The fecundity was determined with data from Nijsse et al. (2015) and Nissen et al. 2013 who approximated the daily faecal output of foxes with 95 g/day (64,6 – 134,9). The range of the production of eggs per female per day was determined for both methods and the average was taken.

### Statistical analysis

Possible correlations between the enteric worm counts and the counted EPGs were assessed using Spearman's correlation coefficient.
The concordance between the outcomes of the McMaster method and the mini-FLOTAC method was assessed with Kendall's Tau-b test.
Associations between the worm count and the EPGs with age, gender or body condition were used assessed using a negative binomial regression model either univariately or multivariately.
The used statistical software was STATA 11 (StataCorp LP, College Station, USA)

## Results

The varying differences in the number of observations per analysis are due to some relevant circumstances that have impaired the quality of the individual sample. For one thing, it was not possible to retrieve all samples from every dissected fox. Some foxes missed their intestines due to damage caused by the impact of the bullet, others seem to have defecated just before being shot, so no EPG could be determined. Furthermore the gender of some worms could not be determined due to their bad condition since they were either decaying for too long or the repeated freezing and thawing and/or the extraction from the intestines caused noticeable damage. For every analysis, only data sets with all relevant data were considered.
From the 78 foxes, 46 to 49 samples were relevant for the correlation between EPG and the worm count with age, gender and BCS, while only 33 could be used for the comparison of the techniques with the worm counts. The differences in counts have a variety of explanations. It was not possible to retrieve faeces from all foxes or the retrieved faeces was so little that only the CSF could be executed.

From some foxes only the faeces was available and from some only the enteric worm count, which explains the low number when correlating the EPG with the worm count.

The Kendall's Tau-b test resulted in a strong positive association between the McMaster and the mini-FLOTAC technique ($r = 0,97$ ; $p < 0.01$) with a concordance of 89%.

The fecundity range per female *T. canis* for the McMaster was 0 – 182.083 eggs / day / female *T. canis* (E/D/F) and the average was 23702 E/D/F, while the range with the mini-FLOTAC was 0 – 149.150 E/D/F with an average of 20519 E/D/F.

Table 1 – Analysis between properties of the foxes and the EPGs with both methods as well as the worm count

| | EPG McM (n=49) | | EPG mF (n=49) | | Worm count (n=46) | |
|---|---|---|---|---|---|---|
| | IRR (95% CI) | p | IRR (95% CI) | p | IRR (95% CI) | p |
| Fox gender | 0,94 (0,26 – 3,39) | 0,92 | 0,94 (0,28 – 3,17) | 0,92 | 1,32 (0,75 – 2,31) | 0,34 |
| Age | 1,1 (0,27 – 4,55) | 0,89 | 1,36 (0,36 – 5,24) | 0,65 | 1,07 (0,60 – 1,90) | 0,82 |
| BCS | 0,92 (0,65 – 1,29) | 0,63 | 0,90 (0,66 – 1,23) | 0,50 | 0,81 (0,69 – 0,96) | **0,01** |

EPG= Eggs per gram faeces, McM = McMaster, mF = mini-FLOTAC, BCS = Body condition score

No significant correlation could be found between the total number of worms retrieved from the intestines and the egg count using with the quantitative techniques, as can be seen in table 2. The positive correlations between the EPGs of the McMaster and mini-FLOTAC with the worm count were not significant, while the positive correlation between the two techniques was highly significant.

Table 2 – Total worm count correlation with McMaster and mini-FLOTAC

| n=33 | Worm count | EPG McM rho | EPG mF rho |
|---|---|---|---|
| Worm count total | - | 0,32 | 0,28 |
| EPG McM p-value | 0,069 | - | 0,97 |
| EPG mF p-value | 0,122 | 0,000 | - |

There was also no significant correlation between the egg counts and the number of worms identified as female, as shown in table 3. The positive correlations here between the EPG and worm count were not significant, but the positive correlation between the techniques was highly significant.

Table 3 – Female worm count correlated with McMaster and mini-FLOTAC

| n=33 | Worm count female | EPG McM rho | EPG mF rho |
|---|---|---|---|
| Worm count female | - | 0,30 | 0,29 |
| EPG McM p-value | 0,090 | - | 0,97 |
| EPG mF p-value | 0,105 | 0,000 | - |

## Discussion

The presented study shows a correlation between the body condition of foxes with their intestinal worm count and a strong concordance between the McMaster and the mini-FLOTAC.
One goal of this study was to look for possible correlations between the fecundity of T. canis in red foxes, the worm burden they represent and parameters of the foxes including age, gender and BCS. A correlation between the BCS and the intestinal worm count was the only significant result in this study. Neither age nor gender were correlated with the EPG or the worm count. The relevant literature showed that other researchers had differing outcomes. Saeed et al. (2006) found in a study on the Danish fox population that male foxes had significantly more often patent infections than female foxes and also showed higher intestinal worm counts than female foxes. A similar result had been found by Franssen et al. (2014). Another study by Stuart et al. (2013) did not find a difference between the frequency of infection in Irish red foxes. From a biological standpoint it is difficult to explain why there should be a difference in prevalence of *T. canis* infections between the genders of the foxes. Both genders supposedly have the same feedings habits, the same environment, the only differences are the hormonal status especially during pregnancy of the female foxes. This solely female circumstance might lead to a ‘depletion’ of somatic larvae from the tissues, as the larvae migrate to the fetuses via the uterus during pregnancy or via the mammae to the cubs after birth. If then a stressor appears in the life of a fox, males have more somatic larvae left to be reactivated, which might lead to more patent infections in male foxes. Nijsse et al. (2016) found a 2,38 fold increase of the hazard ratio for recurring patent infections when corticosteroids were administered to subjects of his study. Endogenous corticosteroids and the accompanying immunosuppression should have a comparable effect on the reactivation of somatic tissue larvae which in turn might lead to more patent infections in male foxes. In this study no significant difference could be found either in the frequency or quantity of the worm burdens between the host genders.
Saeed et al. (2006) also found that the fecundity of female *T. canis* was lowered in adult female foxes compared to male foxes. This finding from Denmark could not be verified for Dutch red foxes in this study. This might be due to the larger sample size and other factors incorporated into the Danish analysis, like different regions, different seasons and different years, which were not part of this study. A factor partly explaining the difference with the Danish study might be the young age of the majority of the Dutch foxes (55 < 1 year vs 23 > 1 year ; 70/30), as they were in the adolescent phase and possibly still developing age-resistance, which might have been in different stages of development in each individual.
As the dissections took place in early 2017, the foxes were shot shortly before. Foxes usually give birth at the end of March and early April and the foxes supplied by the hunters were shot between mid January and the end of March. As a conclusion it is relatively safe to conclude that all the foxes estimated to be younger than one year old had passed the first half year of their lives. The presumably further developed age-resistance puts them more closely to the adult immunity group than to immunologically less equipped cups, but still in development. It has been shown by Nijsse et al. (2014) that dogs between 6 and 12 months of age still have double the chance of a patent infection compared to dogs older than one year. Similar to the results of Stuart et al. (2013) the present study could not provide evidence for a significant difference in EPG or worm count between the two age groups. This might be due to some limiting factors. For one thing, in the present study the comparison between dogs and foxes might be obfuscated by the different feeding habits of the two species, as foxes consume much more paratenic hosts than the average dog. Which in turn can lead to a patent infection without a HTM.

With wild animals, it is relatively difficult to achieve an even sample distribution. In this study we relied on samples provided by hunters the province of Groningen. Their selection was based on the availability of foxes within their hunting grounds. This method of sample selection has some potential biases and might have influenced the outcome of the sampling unintentionally. It seems possible that

they have harvested inattentive or inexperienced foxes. They could unintentionally have included young, sick, injured or otherwise immunologically compromised individuals that were not as alert and therefore more easily shot. There were specimens that had injuries or special circumstances that might have increased the chance of being selected and shot by the hunter. Some of the foxes had a missing eye, (open) fractures of the extremities in different stages of recovery and a few were pregnant. All these additional and not predictable factors considered led to a not ideal composition of the samples and might have implications for the endocrine status of the individuals in question. These altered statuses might have influenced the data, as mentioned earlier.

Another factor that might have shifted the outcomes out of the significant range was the bad condition of the worms in a few samples, which might not have been representative of the individual sample. In some samples the worms were shredded during the extraction from the intestines at the RIVM. Several freeze and thaw cycles, as well as a unintended delay of the processing of the samples didn't help the condition of the worms and led sometimes to a malacia due to a delay in the workflow. These factors resulted in a relatively high number of worms that couldn't be included in the data as they were torn in multiple parts or the gender could not be determined due to malacia or adhesions.

The other goal of this study was to compare the two techniques McMaster and mini-FLOTAC. The result shows a strong and highly significant positive association between these methods, suggesting that the mini-FLOTAC is an accurate alternative to the McMaster technique.

With the mini-FLOTAC the searched volume of fluid is 6,66 times larger, the grid with 24 rows spread over the two chambers is double the size compared to the 12 rows with the McMaster. The grid rows of both techniques have the width to be examined with 100x magnification. The larger grid and the bigger volume of the mini-FLOTAC results in a higher resolution compared to the McMaster. The higher resolution helps when overall lower EPGs are to be expected from an intestinal parasite and a quantitative technique is wanted or needed. While the volume is larger, it also takes significantly longer to search the double sized grid after the same waiting period as with the McMaster technique. Van den Putte et al. (2016) and Barda et al. (2014) found that the time to count a grid with the McMaster takes about 7 minutes, while the time for the larger grid of the mini-FLOTAC is about 13 minutes. These few minutes of more time spent are rewarded with a much lower detection threshold. The concordance of 89% between both quantitative techniques has been matched by results from Van den Putte et al. (2016) who found a correlation coefficient of 90% with a p-value of 0,001 for the comparison of the methods. These outcomes seem to be sufficient to use the mini-FLOTAC as an alternative for every day use in the veterinary practice. Especially in applications that ask for a lower detection threshold without the immediate availability of a centrifuge. Although there is a chance for a false positive diagnosis due to coprophagy in dogs, the lower threshold helps to detect potential infections even earlier. (Deplazes et al. 2011).

However, this finding needs to be considered within some limitations as well. Both methods suffered from not fresh and repeatedly frozen faeces. Some *T. canis* eggs were dwelling on the bottom of the counting chambers instead of rising to the surface, as they should have. This either due to damage from the freezing or osmotic pressure from the used sucrose solution or a combination thereof, as well as the high viscosity of the sugar solution, that might have impeded some (already damaged?) eggs from rising in time. Although the specific gravity should have been sufficient (Dryden et al. 2010). On the other hand, in single instances eggs couldn't be detected with the CSF, but could be found in low numbers with the quantitative techniques. This either due to user error during the CSF, like not perpendicularly removing the coverslip from the tube or spillage during this action. The quantitative techniques were used on these initially negative samples because *Capillaria* spp were detected in the CSF, which were part of a different study.

When the subjective usability of both methods is compared, the mini-FLOTAC lacks behind the McMaster technique. The process of filling the chambers of the mini-FLOTAC at an adequate angle without introducing air bubbles is the first step to be mastered. After the waiting period the second pitfall is the turning of the upper disc. If this is done with too much or too little pressure or if it is the first run of the day without a liquid layer in between the discs, the chance of introducing big air bubbles in the chambers is quite high. When the discs are wetted before the first usage of the day it lowers the risk of big bubbles during the turning of the discs. If these points are considered the mini-FLOTAC is just as easy to use as the McMaster.

## Conclusion

In conclusion, the study was able to demonstrate that a low BCS is associated with a high worm burden in Dutch red foxes. The fecundity of the *T. canis* in Dutch red foxes has been established within the ranges known from literature.

The mini-FLOTAC has proven to be a reliable and accurate technique with a lower detection limit than the McMaster, but with a little more finesse needed for handling. It is suitable for everyday use in the veterinarian practice with an emphasis on intestinal parasites that shed few eggs.

## References


Barda, Beatrice et al. 2014. “Mini-FLOTAC, Kato-Katz and McMaster: Three Methods, One Goal; Highlights from North Argentina.” *Parasites and Vectors* 7(1):1–7.

Cringoli, Giuseppe et al. 2017. “The Mini-FLOTAC Technique for the Diagnosis of Helminth and Protozoan Infections in Humans and Animals.” *Nature Protocols* 12(9):1723–32.

Deplazes, Peter, Frans van Knapen, Alexander Schweiger, and Paul A. M. Overgaauw. 2011. “Role of Pet Dogs and Cats in the Transmission of Helminthic Zoonoses in Europe, with a Focus on Echinococcosis and Toxocarosis.” *Veterinary Parasitology* 182(1):41–53. Retrieved (http://dx.doi.org/10.1016/j.vetpar.2011.07.014).

Despommier, Dickson. 2003. “Toxocariasis : Clinical Aspects , Epidemiology , Medical Ecology , and Molecular Aspects Toxocariasis : Clinical Aspects , Epidemiology , Medical Ecology , and Molecular Aspects.” *Society* 16(2):265–72.

Dryden, M. W., P. A. Payne, R. Ridley, and V. Smith. 2010. “Comparison of Common Fecal Flotation Techniques for the Recovery of Parasite Eggs and Oocysts.” *Veterinary Therapeutics* 6(1):15–28.

Fahrion, A. S., S. Staebler, and P. Deplazes. 2008. “Patent Toxocara Canis Infections in Previously Exposed and in Helminth-Free Dogs after Infection with Low Numbers of Embryonated Eggs.” *Veterinary Parasitology* 152(1–2):108–15.

Ferreira, Ana et al. 2017. “Urban Dog Parks as Sources of Canine Parasites: Contamination Rates and Pet Owner Behaviours in Lisbon, Portugal.” *Journal of Environmental and Public Health* 2017.

Franssen, Frits et al. 2014. “Increase in Number of Helminth Species from Dutch Red Foxes over a 35-Year Period.” *Parasites & Vectors* 7(1):166. Retrieved (http://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-7-166).

Morgan, E. R., D. Azam, and K. Pegler. 2013. “Quantifying Sources of Environmental Contamination with Toxocara Spp. Eggs.” *Veterinary Parasitology* 193(4):390–97. Retrieved (http://dx.doi.org/10.1016/j.vetpar.2012.12.034).

Nijsse, R., H. W. Ploeger, J. A. Wagenaar, and L. Mughini-Gras. 2014. “Toxocara Canis in Household Dogs: Prevalence, Risk Factors and Owners??? Attitude towards Deworming.” *Parasitology Research* 114(2):561–69.

Nijsse, Rolf, Lapo Mughini-Gras, Jaap A. Wagenaar, Frits Franssen, and Harm W. Ploeger. 2015. “Environmental Contamination with Toxocara Eggs: A Quantitative Approach to Estimate the Relative Contributions of Dogs, Cats and Foxes, and to Assess the Efficacy of Advised Interventions in Dogs.” *Parasites & Vectors* 8(1):397. Retrieved (http://www.parasitesandvectors.com/content/8/1/397).

Nijsse, Rolf, Lapo Mughini-Gras, Jaap A. Wagenaar, and Harm W. Ploeger. 2016. “Recurrent Patent Infections with Toxocara Canis in Household Dogs Older than Six Months: A Prospective Study.” *Parasites and Vectors* 9(1):1–11. Retrieved (http://dx.doi.org/10.1186/s13071-016-1816-7).

Nissen, Sofie et al. 2013. “Population Dynamics and Host Reactions in Young Foxes Following Experimental Infection with the Minute Intestinal Fluke, Haplorchis Pumilio.” *Parasites and Vectors* 6(1):2–11.

Overgaauw, P. A. M. 1997. “Generall Introduction Aspects of Toxocara Epidemiology, Toxocariosis in Dogs and Cats.” *Critical Reviews in Microbiology* (April 1994):147–56.

Van Den Putte, N., E. Claerebout, and B. Levecke. 2016. “Evaluatie van de Mini-FLOTAC-Methode Voor de Detectie van Gastro-Intestinale Parasieten Bij Grote Huisdieren.” *Vlaams Diergeneeskundig Tijdschrift* 85(1):15–22.

Richards, D. T. and J. W. Lewis. 2018. “Fecundity and Egg Output by Toxocara Canis in the Red Fox, Vulpes Vulpes.” (2001):157–64.

Saeed, I. S. and C. M. O. Kapel. 2006. “Population Dynamics and Epidemiology of Toxocara Canis in Danish Red Foxes.” *Journal of Parasitology* 92(6):1196–1201.

Saeed, Isam, Kensuke Taira, and Christian M. O. Kapel. 2005. “Toxocara Canis in Experimentally Infected Silver and Arctic Foxes.” *Parasitology Research* 97(2):160–66.

Stuart, Peter et al. 2013. “A Coprological Survey of Parasites of Wild Carnivores in Ireland.” *Parasitology Research* 112(10):3587–93.