Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands

Paul A.M. Overgaauw a,*, Linda van Zutphen b, Denise Hoek c, Felix O. Yaya d, Jeroen Roelfsema c, Elena Pinelli c, Frans van Knapen e, Laetitia M. Kortbeek c

a RNA BV, Yalelaan 2, 3584 CM, Utrecht, The Netherlands
b Quantitative Veterinary Epidemiology Group, Wageningen Institute of Animal Sciences, Wageningen University, The Netherlands
c National Institute for Public Health and the Environment, Department of Parasitology, Laboratory for Diagnosis and Screening of Infectious Diseases, Bilthoven, The Netherlands
d Free University of Amsterdam, Department of Biomedical Sciences, The Netherlands
e Institute for Risk Assessment Sciences, Division of Veterinary Public Health, Utrecht University, The Netherlands

1. Introduction

In The Netherlands, the number of pets has increased during the last decade, of which 1.8 million (M) dogs, 3.3 M cats, and 0.9 M rabbits are mostly responsible. Stray cats are common; however, stray dogs are hardly present in the Netherlands. The number of households with pets increased from 50% in 1999 to 55% in 2005 (Dutch Council for Animal Affairs, 2006).

As pets are increasingly considered a member of the family, physical contact is very common. Companion animals enhance the psychological and physiological well-being of the human. Dogs play an important role in the development and in the treatment of behavioral

ABSTRACT

Pets may carry zoonotic pathogens for which owners are at risk. The aim of the study is to investigate whether healthy pets harbour zoonotic parasitic infections and to make an inventory of the interactions between pet-owners and their companion animals in the Netherlands. Fecal and hair samples were collected from healthy household dogs and cats in Dutch veterinary practices. Owners were interviewed about interaction with their pets. The samples were investigated by microscopy, ELISA, and PCR.

From 159 households, 152 dogs (D) and 60 cats (C), information and samples were collected and examination for several zoonotic parasites was performed. Toxocara eggs were found in 4.4% (D) and 4.6% (C) of the fecal samples and in 12.2% (D) and 3.4% (C) of the fur samples. The median epg in the fur was 17 (D) and 28 (C) and none of these eggs were viable. From 15.2% of the dog and 13.6% of the cat feces Giardia was isolated. One canine and one feline Giardia isolate was a zoonotic assemblage A (12%). Cryptosporidium sp. were present in 8.7% (D) and 4.6% (C) of the feces.

Fifty percent of the owners allow the pet to lick their faces. Sixty percent of the pets visit the bedroom; 45–60% (D–C) are allowed on the bed, and 18–30% (D–C) sleep with the owner in bed. Six percent of the pets always sleep in the bedroom. Of the cats, 45% are allowed to jump onto the kitchen sink. Nearly 39% of the dog owners never clean up the feces of their dog. Fifteen percent of the dog owners and 8% of the cat owners always wash their hands after contact with the animals. Close physical contact between owners and their pets is common and poses an increased risk of transmission of zoonotic pathogens. Education of owners by the vet, specifically about hygiene and potential risks, is required.

* Corresponding author. Tel.: +31 342 419798; fax: +31 342 419794. E-mail address: p.overgaauw@planet.nl (Paul A.M. Overgaauw).

0304-4017/$ – see front matter © 2009 Elsevier B.V. All rights reserved.

Please cite this article in press as: Overgaauw, P.A.M., et al., Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. Vet. Parasitol. (2009), doi:10.1016/j.vetpar.2009.03.044
problems of children, the well-being of the elderly, and decrease work leave through illness and visits to the doctor (Beck and Meyers, 1996). Next to the benefits of pets for the human population, there are also potential health hazards associated with the ownership of a pet. Besides the risk of bites, scratches and allergies, several infections can be transmitted to the human as zoonosis. Significant parasitic zoonoses of dogs and cats prevalent in The Netherlands are *Toxocara*, *Giardia*, *Cryptosporidium* and *Toxoplasma* (Buijs, 1993; Overgaauw, 1997a; Valkenburgh et al., 2007). These parasites have an oro-fecal transmission cycle and humans can be infected either by fecal contamination of food, water or the environment (gardens, sandpits and playgrounds) or by direct contact (Good et al., 2004; Hill et al., 2000; Robertson and Thomson, 2002; Smith et al., 2007). Direct contact with dogs and cats that harbour a patent *Toxocara* infection is usually not considered a risk, since the eggs need to mature several weeks before they are infective (Overgaauw, 1997b; Overgaauw and Van Knapen, 2000, 2004).

Recent studies, however, indicate the fur of these pets as another important source of embryonated *Toxocara* eggs (Wolfe and Wright, 2003; Roddie et al., 2008; Aydenizöz-Özkayhan et al., 2008). *G. duodenalis* is known to exhibit species complexity as it has been isolated from humans and animals, including pets (Eligio-García et al., 2005). Companion animals are most often infected with host-specific *Cryptosporidium* sp. from which *C. felis* and *C. canis* infrequently are reported to infect humans (Pedraza-Díaz et al., 2001; Fayer, 2008; Wielinga et al., 2008; Xiao and Feng, 2008), despite close and widespread contact. Therefore, their role in the transmission of human cryptosporidiosis appears quite limited (Xiao and Fayer, 2008).

In the present study, the results are presented from the examination of several zoonotic parasites in fecal and fur samples from healthy household dogs and cats. In addition, an inventory of the interactions between pet-owners and their companion animals in the Netherlands is reported.

2. Materials and methods

2.1. Questionnaires

Ten veterinary clinics in urban and rural areas in different provinces in The Netherlands were visited weekly during the period of March–May 2007. Owners of clinically healthy dogs and cats were asked to participate in the study and then to fill in a questionnaire. In this way information was collected of the owners, the animals, the physical relationship between pet-owners and their pets, and management factors such as nutrition (raw vs. commercial food), defecating location cat (outdoor, cat litter box, or both), cleaning frequency of the litter box, walking environment dog (urban, rural and dog exercising area), and deworming frequency. Finally information regarding fur length, body weight, living environment, location, breed, and cat outdoor or indoor access was questioned.

2.2. Egg recovery technique from hair

With the pet-owner consent, fur samples were collected by combing the dorsum (lumbar and sacral area) and flanks, representing the areas where owners normally pet their animals. Combs were cleaned and every time disinfected with a halogenated organic compound (Neosabeny1®) after use. At the moment of analysis, there was no standardized and validated method published for the isolation of *Toxocara* eggs from dog and cat hair. Therefore the following method was developed and validated using *T. canis* eggs available at the Department of Parasitology. Different numbers (8, 39, and 100) of *Toxocara* eggs were added separately to hair samples from a *Toxocara*-negative dog. To recover back the *Toxocara* eggs, the spiked hairs were washed and shaken vigorously as described below. The overall recovery rate for this method was 95%.

The hair sample was weighted and samples with a weight less than 0.0025 g were not examined, as they were considered too small. A soap solution of three drops (approximately 0.2 ml) of the detergent Tween 20 (Merck, Germany) in 40 ml sterile distilled water was prepared to wash the hair by shaking vigorously and allowing to stand for a minimum of 10 min. The floating hair was transferred with a pair of tweezers to another tube and washed again with 40 ml phosphate buffer solution (PBS). The suspension was allowed to stand for a minimum of 10 min and the hairs were discarded. The suspensions from the two tubes were centrifuged at 800 × g for 10 min and the supernatant decanted until approximately 1 ml. The sediments from the tubes were re-suspended and transferred to combine the pellet suspension in a tube using a sterile pipette. The tubes were flushed once with drops of PBS and combined. This tube was centrifuged again at 800 × g for 10 min and the supernatant again decanted until approximately 1 ml. The sediment was re-suspended and transferred to a 1.5 ml Eppendorf tube. Finally, this tube was centrifuged at 800 × g for 10 min and supernatant discarded till approximately 100 µl. The sediment was re-suspended and the whole volume examined using a light microscope under 400× magnifications. Positive samples for *Toxocara* eggs were preserved in 0.05 M sulphuric acid solution to allow embryonation, as described by De Savigny (1975), for a viability test at a later point in time.

2.3. Confirmation of isolated *Toxocara* eggs from the fur

The *Toxocara* eggs found in the fur were identified using a PCR with primers NC2 and Tcan1 or NC2 and Tcat1 (Jacobs et al., 1997).

2.4. Determination of the viability of isolated *Toxocara* eggs

The hair solution samples with *Toxocara* eggs stored in 0.05 M sulphuric acid were retrieved from storage at room temperature after 6 weeks. The suspension of *Toxocara* eggs was centrifuged at 800 × g for 10 min and the supernatant removed. The suspension was examined by light microscopy at 100–400× magnification. The eggs were classified as non-viable (empty, not intact egg wall), unembryonated or fertilized (intact egg wall, with contents), embryonated (with cell divisions), or infective (with larva).
2.5. Fecal examination

Fecal samples were provided in a feces container by the owner. The samples were sent to the laboratory within 24 h of collection and stored at 4°C until analysis.

An ELISA was used (ProSpecT, Alexon-Trend, USA), according to the manufacturer’s instruction, to determine the presence of Giardia specific antigen (GSA 65 protein) in fecal samples, because with microscopy alone it is possible to miss Giardia cysts from a single fecal sample (Mank et al., 1997).

An ether-sedimentation flotation-centrifugation (Ridley and Hawgood, 1956) method was used to identify nematode parasite eggs and cysts in the fecal samples. The samples were examined by light microscopy with 100–400× magnifications.

A modified (cold method) Ziehl–Neelsen staining (Henriksen and Pohlenz, 1981) was performed on a light smear from the Ridley’s concentration, after air drying overnight, to identify oocysts of Cryptosporidium. The slide was examined by light microscopy with oil immersion lens at 1000× magnification.

2.6. Genotyping of Giardia and Cryptosporidium

Immediately after a positive ELISA for Giardia in fecal samples, the cysts were isolated from feces using Dynabeads that are covered with antibodies directed against Giardia cysts. DNA was isolated from the cysts after freeze/thawing using the Puregene Kit (BIOZYM). Isolation of Giardia cysts and subsequent DNA isolation was performed to identify the genotype by PCR and direct sequence (Van der Giessen et al., 2006).

Genotyping was performed with 18S small subunit gene (Van der Giessen et al., 2006). The 18S gene amplification was performed using the primers 18S-1 and 18S-A in a 50 μl reaction mixture containing 10 μM dNTP, 10× buffer II, 25 mM MgCl2, 5 U/μl Ampli Taq (Applied Biosystem, The Netherlands). The PCR was carried out with the following condition: one cycle of 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 3 min.

Purified (Qiagen/Westburg, Leusden, The Netherlands) PCR products were sequenced in both directions using the BigDyeTerminator kit (Applied Biosystems, Nieuwekerk a/d IJssel, The Netherlands) and an ABI automated sequencer. DNA sequences from this study were compared with DNA sequences from the reference strain from Zoopnet (RIVM, Bovenspletenagel, The Netherlands). Multiple alignments were made using ClustalW. PCR on Cryptosporidium positive fecal samples was performed on the 18S small subunit gene according to Wielinga et al., 2008. DNA was isolated on three separate occasions: once with the NucliSens kit (BioMerieux Benelux B.V. Boxtel), and two times with the Hi Pure Kit (Roche, Almere, The Netherlands).

2.7. Statistical analysis

For the statistical analysis, three steps in SAS (SAS 9.1.3, © 2002–2004, SAS Institute Inc., Cary, NC, USA) were performed. First, a descriptive analysis was performed to determine the prevalence of the studied pathogens. The distribution of the animals over the classes of all management factors was determined, as well as the prevalence of the pathogens per class of each management factor. Secondly, a univariable logistic regression analysis was done with binomially distributed outcome data to determine effects of the management factors on infection with the studied parasites. In the model, the dependent variable was the presence or absence of infection, and the independent variable was the management factor. Odds ratios and their 95% confidence intervals have been calculated using exact logistic regression.

3. Results

3.1. Sampling and questionnaires

With the pet-owner consent, from 159 households in total 148 dog and 59 cat fur samples were collected, and from 92 dogs and 22 cats fecal samples were provided by the owner.

Questionnaire results show that half of the owners allow the pet to lick their faces. Sixty percent of the pets visit the bedroom; 45% of the dogs and 62% of the cats are allowed on the bed, and 18% and 30% of the dogs and cats, respectively, are allowed to sleep with the owner in bed. Of the cats, 45% are allowed to jump onto the kitchen sink. Most cats are allowed to go outside, but 32% stay indoors and accordingly use the litter box for defection. From the outdoor cats, 76% (32/41) also use the litter box. Fifty-five percent of owners clean the litter box more often than twice a week, while 39% of the dog owners never clean up their dogs’ feces. Forty-five percent of the dogs and 40% of the cats were dewormed more often than twice a year. Fifteen percent of the dog owners and 8% of the cat owners always wash their hands after contact with the animals.

3.2. Hair analysis

The results of the analysis of all fecal and fur samples from in total 224 investigated dogs and cats, originating from 159 pet-owners, are presented in Table 1. The average weight of the fur samples, collected after thorough combing, was 0.129 g for the dog and 0.037 g for the cat (range: 0.0026–0.4725). In the fur of 18 dogs (12.2%) and two cats (3.4%) Toxocara eggs were found. The average number of eggs per sample was 3.5 (1–31) and recalculating to eggs per gram fur (epg) resulted for the dogs in a mean epg of 94 (3.8–1065) and a median epg of 17. For the cat a mean as well as median epg of 28 (22.9–32.5) was found. The eggs in five examined samples were fertilized (25%), except one egg (from the sample of 31 eggs) and measured from 65–80 μm. Out of the 20 samples there was sufficient remaining material of only five samples to perform PCR and these eggs were confirmed as Toxocara. From the remaining samples not enough material could be extracted to perform PCR for the confirmation of these eggs as Toxocara. The viability of the eggs was examined after 6 weeks culturing in sulphur acid solution. None of these eggs were viable. Positive canine hair samples for Toxocara eggs originated from different breeds and fur length: seven
short, nine medium and two long hair breed. The average age of the dogs was 6.5 years (0.5–13). From nine dogs with egg-positive hair samples, the feces were also investigated. These all tested negative for intestinal parasites. From the two cats, one had no outdoor access.

3.3. Fecal analysis

Based on the fecal analysis with the Ridley concentration method, four dogs and one cat were positive for T. canis (Table 1). Fourteen dogs and three cats were positive for Giardia and eight dogs and one cat for Cryptosporidium. One dog showed a double infection of Giardia with T. canis and two other dogs Giardia with Cryptosporidium. All three dogs with multiple infections originated from the same household. Next to these animals there was one other dog double infected with Cryptosporidium and Giardia. There were no Toxoplasma cysts found in the cat feces.

3.4. Genotyping of Giardia and Cryptosporidium

The Giardia fecal examination with Ridley’s concentration method resulted in 17 positive samples. With the enzyme linked immunosorbant assay (ELISA) 19 positive (16 dogs and 3 cats) samples were amplified by PCR for 18S rDNA. The 18S rDNA positive (16 dogs and 3 cats) were found. DNA isolates of these samples were genotyped by the enzyme linked immunoabsorbant assay (ELISA) 19 positive samples, the feces were also investigated. These all tested negative for intestinal parasites. From the two cats, one had no outdoor access.

3.4. Genotyping of Giardia and Cryptosporidium

The Giardia fecal examination with Ridley’s concentration method resulted in 17 positive samples. With the enzyme linked immunosorbant assay (ELISA) 19 positive (16 dogs and 3 cats) samples were amplified by PCR for 18S rDNA. The 18S rDNA positive (16 dogs and 3 cats) were found. DNA isolates of these samples were genotyped by the enzyme linked immunoabsorbant assay (ELISA) 19 positive samples, the feces were also investigated. These all tested negative for intestinal parasites. From the two cats, one had no outdoor access.

The genotype was determined from the 18S DNA sequences alignment on ClustalW and the analysis of five single nucleotide polymorphisms (SNPs). With the 18S sequences the isolates were grouped into assemblage A (two samples), assemblage C (seven samples), assemblage D (three samples), assemblage C/D (one sample), assemblage F (one sample), and one sample with an unknown assemblage. The unknown assemblage had two ambiguous nucleotide bases/codes (Y and M) (Table 2).

Genotyping the Cryptosporidium positive samples, determined after the Ziehl-Neelsen staining, proved to be impossible. PCR yielded no results.

3.5. Risk factors

With logistic regression no significant difference was found for Giardia, Cryptosporidium and Toxocara (in feces) infection between dogs and cats.

Only for Toxocara eggs in the fur, an almost significant higher prevalence in dogs compared to cats was found ($p = 0.08$). Toxocara eggs were four times more present in dog than in cat fur.

Some management factors concern only dogs or cats. A subsequent logistic regression analysis was performed to determine effects of management factors on Toxocara in fur, Toxocara in feces, Toxascaris, Cryptosporidium, and Giardia infection. Unfortunately the number of animals with an infection was too low to perform valid statistical analyses except for Toxocara in fur. In a univariate analysis it was found that dogs off the leash or both on and off the leash when outdoors have significantly more often (OR 8.7) Toxocara eggs in their fur than dogs that are always on the leash ($p = 0.03$). For the variables fur length and body weight of the dogs there was no significant effect found ($p > 0.25$).

4. Discussion

Patent Toxocara infections in dogs and cats are considered as a public health risk because of their zoonotic potential. In the literature the presence of stray dogs, the growing popularity of pet dogs and cats, the limited open green areas, pica and geophagia by children, and the close physical contact between owner and pet are given as a risk of transmission (Overgaauw, 1997a; Smith et al., 2006).

The prevalence of Toxocara infections in household dogs and cats, based on fecal egg output, in our study is in agreement with the earlier reported 3–5% in The Netherlands (Overgaauw, 1997c) and Belgium (Claerebout et al., 2005). In stray animals or animals in animal shelters, prevalences may be higher, up to 9% in dogs (Le Nobel et al., 2004) and 28% in cats (Robben et al., 2004).
Regarding *Toxocara* eggs in hair, a few other studies in dogs were carried out in the UK and Ireland (Wolfe and Wright, 2003; Roddie et al., 2008), France (Regosz, 2007), and Turkey (Aydenizöz-Ozkayhan et al., 2008). Cat fur was investigated in Germany (Hasslinger et al., 1973) and France (Regosz, 2007). An overview of these studies is presented in Table 3. Because the mean epg is easily influenced by outliers, the median epg (if determined) is also presented.

We developed a practical and validated egg recovery technique from hair, without the use of time-consuming sieves. The overall recovery rate, however, is high. With this technique *Toxocara* eggs were found in the hair of 12% of the investigated dogs and 3% of the cats. An explanation of the significantly higher number of *Toxocara* eggs in the dog fur could be explained by the fact that dog (playing) behavior results in more soil contact. Cats, moreover, lick their fur more intensively and thus may find it easier to remove any present eggs. In dogs the eggs were found in all fur types and nearly all dogs were adults. There was no simultaneous *Toxocara* infection in the feces. Therefore, the presence of eggs in the fur does not necessarily mean that this is always a result of self-contamination. This is also the conclusion for the one cat with contamination of its hair, but without outdoor access. A possible explanation may be that the eggs were acquired through transmission for instance via the shoes of the owner. The presence of eggs in house dust was established in a survey among dog breeders (Overgaauw and Boersema, 1998).

The presence of *Toxocara* eggs in the fur, especially in puppies and stray dogs, is an indication that direct contact with these animals may put humans at risk for developing an infection of clinical toxocarosis. There are two conditions that have to be fulfilled before an infection can develop.

First, the eggs need to be embryonated to be infective. None of the eggs found in our study were viable, probably a result of UV-light influence and/or lack of humidity. Also, the fact that the eggs were probably obtained from the environment may be an explanation. Development to an infective stage will last 3–6 weeks to several months, depending on soil type and climatic conditions (time of the year), such as temperature and humidity. In the other studies a low percentage of the eggs were embryonated.

Secondly, ingestion of a sufficient number of eggs is required to stimulate an immune response and to cause clinical human toxocarosis. Antibody levels in dogs and mice were found to be strictly dose-related (Glickman and Schantz, 1981; Kayes et al., 1985; Pinelli et al., 2007) and mice were found to be strictly dose-related (Glickman and Schantz, 1981; Kayes et al., 1985; Pinelli et al., 2007) and this may also be the case in man. In most of the mentioned studies, hair samples were collected from the perianal area after thorough combing, and even then not more than one egg was found per sample. This, together with the fact that *Toxocara* eggs are very sticky, and therefore difficult to remove from the coat of a dog or cat, makes ingestion of sufficient numbers of eggs unlikely. It is, however, remarkable in this context to notice that in the study of Roddie et al. (2008) some cases were presented with very high number of eggs in the hair of stray dogs, and mainly their puppies, without significant difference between the sampled perianal area and the dorsal region.

In some reports it has been suggested that the epg of *Toxocara* in fur of infected animals is much higher than in soil (Wolfe and Wright, 2003, 2004; Roddie et al., 2008). This is undoubtedly the case, but does this suggest that dogs and cats infected with *Toxocara* may infect people by direct contact and provide a better explanation of the epidemiology of the disease? Even in the worst case scenario of highly contaminated fur, e.g. with the highest *Toxocara* epg of 300 and an embryonated rate of 4% from the study of Wolfe and Wright (2003), it is necessary to ingest more than 4 g of hair, with 12 embryonated eggs per gram, to ingest 50 infective eggs. As example Fig. 1 shows the largest hair sample from our study with a weight 0.47 g to indicate roughly the size of a few grams.

The hair contamination with *Toxocara* eggs, found in the studies of Wolfe and Wright (2003) and Roddie et al. (2008), is used to suggest that this is probably the main route of transmission to the human and that there lacks sufficient evidence of a direct link between seroprevalence in people and soil contamination (sapro-zoonosis). There are two arguments to disprove this suggestion. Many studies reported that children are more frequently infected than adults and that VLM with more severe clinical symptoms is mainly found in children of 1–3 years of age (Glickman and Schantz, 1981; Van Knapen et al., 1983, 1992; Schantz, 1989). This can be explained because young children often play in and have closer contact with soil samples.

### Table 3

Comparable studies of *Toxocara* fur infection in dogs and cats.

<table>
<thead>
<tr>
<th>References</th>
<th>Species</th>
<th>Nr. tested</th>
<th>Prevalence</th>
<th>Median epg</th>
<th>% Embryonated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hasslinger et al. (1973)</td>
<td>Private/stray cats</td>
<td>17</td>
<td>1(6%)</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>Wolfe and Wright (2003)</td>
<td>Private/farm/shelter dogs</td>
<td>60</td>
<td>15(25%)</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>Regosz (2007)</td>
<td>Private dogs</td>
<td>29</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Private cats</td>
<td>9</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Stray cats</td>
<td>7</td>
<td>1(14%)</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>Roddie et al. (2008)</td>
<td>Adult stray dogs</td>
<td>75</td>
<td>42(56%)</td>
<td>7</td>
<td>0.1%</td>
</tr>
<tr>
<td></td>
<td>Puppy stray dogs</td>
<td>25</td>
<td>25(100%)</td>
<td>793</td>
<td>0.3%</td>
</tr>
<tr>
<td>Aydenizöz-Ozkayhan et al. (2008)</td>
<td>Private dogs</td>
<td>51</td>
<td>11(22%)</td>
<td>10</td>
<td>8%</td>
</tr>
<tr>
<td>This study</td>
<td>Private dogs</td>
<td>148</td>
<td>18(12%)</td>
<td>17</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Private cats</td>
<td>59</td>
<td>2(3%)</td>
<td>28</td>
<td>0%</td>
</tr>
</tbody>
</table>

* Not determined.
potentially contaminated soil in yards and sand-pits. In addition, they put their fingers into their mouth, sometimes eat dirt (pica), and are not very hygienic (Holland et al., 1995). About 40% of patients with Toxocara ocular larva migrans showed a history of pica (Schantz et al., 1979). Such a high percentage of children of this age are not likely to have intense direct contact with (stray) dogs and cats to retrieve enough embryonated eggs from the fur to cause clinical disease.

Another argument is the fact that some surveys did not support a relationship of human toxocarosis and exposure to dogs or cats in the household (Buijs, 1993; Woodruff et al., 1978). The relationship of the presence of (young) dogs in households and toxocarosis (Schantz et al., 1979, 1980; Worley et al., 1984) or the presence of Toxocara antibodies (Glickman et al., 1981; Holland et al., 1995) has been described, but did not provide a reliable indication that these dogs or cats were the sources of the infections. Although in the present study anti-Toxocara antibodies were not measured in dog owners, we do not consider stroking a dog or cat a risk for acquiring a Toxocara infection. However, the results from different studies reveal the need for sufficient deworming schedules, especially in puppies.

Giardia has been isolated from humans and animals. This has raised the issue as to whether G. duodenalis is a zoonosis. In a study in the Netherlands among gastro-enteritis patients from general practitioners, Giardia was found in 5.4% of cases and in 3.3% of controls. There was no correlation between protozoal diarrhea and the ownership of pets (De Wit et al., 2001). Assemblage A has been found in humans, livestock, cats and dogs and assemblage B in humans, dogs, and cats. Both assemblages seem to be related with clinical symptoms in humans (Homan and Mank, 2001). Other genetic assemblages within the G. duodenalis group appeared to be confined to specific animal hosts, like cats (assemblage F) and dogs (assemblages C and D). In a study in the Netherlands, human and animal assemblages A and B Giardia-isolates could be identified. However, phylogenetic analysis revealed different sub-clustering for human and animal isolates, where host-species-specific assemblages (C–G) could be identified (Van der Giessen et al., 2006). In our study 14% of the cat fecal samples and 15% of the dog samples tested positive for G. duodenalis. Most isolates were of the assemblages C and D showing species specificity. The human isolate assemblage A detected in one dog and one cat sample is zoonotic and the first Dutch detection; until now, genotyping of Giardia isolates from fecal samples specific for household pets (dogs and cats) was not previously reported in The Netherlands. Studies done in the past on the genotype of Giardia in Dutch human patients and some animal isolates suggested that the genotype of G. duodenalis is species-specific, except for the human genotypes which have been isolated from other animals (Ten Hove et al., 2007; Van der Giessen et al., 2006). These findings were in agreement with earlier findings by Monis and Thompson (2003). In Belgium, 6.4% of 273 privately owned healthy dogs tested positive for Giardia infection; the infection was significantly more prevalent in dogs younger than 1 year. Genotyping resulted in assemblage A in 19/23 dogs (83%) and assemblage D in 17% of the dogs (Claerebout et al., 2005). Further research is needed to establish that pets can be the source of human infection.

The knowledge of the clinical presentation caused by Cryptosporidium sp. is changing because it is possible to type different strains: C. parvum and C. hominis. The latter spread only between humans and the major reservoir of C. Parvum is domestic livestock (Hunter and Thomson, 2005). However, human cryptosporidiosis in relation with dogs or cats (C. canis and C. felis), is very rare (Xiao and Feng, 2008) and not found to be a risk factor in England (Goh et al., 2004). In the Netherlands, of human isolates 1% C. felis was found (Wielinga et al., 2008).

From the results of the parasitic pathogens, this study should be considered as an initial one and a successive study can be performed where infections of human patients with pets are involved and can be used for further risk analysis.

Toxoplasma cysts were not found in the cat feces in this study. Cats only shed Toxoplasma oocysts once during their lifetime, and only during a limited period of time (2–3 weeks) when they have lost their passive immunity and have started to hunt or to eat raw meat (ingestion of tissue cysts). Most of the spontaneous shedding cats found in surveys are younger than 6 months of age. During the excretion period they build up enough immunity to resist a new infection (Dubey, 1995). In the Netherlands 60% of all 1-year-old cats have passed this period, thus they do not longer play an important role in the epidemiology of toxoplasmosis (Van Knapen, 1993, unpublished data) the chance to find a cat that is shedding Toxoplasma cysts is therefore very limited. A validated PCR for Toxoplasma in cat feces was not available and is therefore not used.

Regarding the hygiene of owners in their physical relationship with their pets, Westgarth et al. (2008) investigated the nature and frequency of contacts between dogs and owners in a UK area. Similar results were found in this survey regarding licking the face of the household members (60%), sleeping in the bedroom (33%) and on the human’s bed (14%). The close contacts and sharing of beds were considered to allow transmission of zoonotic diseases or parasites. The percentages of UK dog owners who said...
they pick up their dog’s feces during walks in various environments are impressive compared with the results that we found in The Netherlands. Eighty percent or more of the UK-owners said they do this usually or always in the street, park area or on public pathways.

5. Conclusions

Nowadays, pets enjoy great freedom when inside, which enables transmission of zoonotic pathogens to surfaces and rooms accessible to humans and animals.

Several clinically healthy dogs and cats in this study were infected with potential zoonotic parasites. The prevalences may even be underestimated because several parasites intermittently shed eggs or oocysts. Since no data was available on the infection status of the owners, no conclusions can be drawn about the risk for transmission of these pathogens. Transmission of parasites or protozoa to the human is only possible if the parasites have been developed into the infective stage, are pathogenic for humans (e.g. assemblage A for *G. duodenalis*), and ingestion of sufficient numbers of infective agents has taken place.

Acknowledgements

We thank the students Danielle Resink, Janne Teninga, Renée van de Ven and Kirsten Peeters for their help in collecting the materials in the veterinary practices. The study was funded in part by a research grant from the Foundation for the Dutch Cat Fancy and Foundation Kattenzorg, The Hague.

References


**G Model**

**VETPAR-4790; No of Pages 8**

P.A.M. Overgaauw et al. / Veterinary Parasitology xxx (2009) xxx–xxx

they pick up their dog’s feces during walks in various environments are impressive compared with the results that we found in The Netherlands. Eighty percent or more of the UK-owners said they do this usually or always in the street, park area or on public pathways.

5. Conclusions

Nowadays, pets enjoy great freedom when inside, which enables transmission of zoonotic pathogens to surfaces and rooms accessible to humans and animals. Several clinically healthy dogs and cats in this study were infected with potential zoonotic parasites. The prevalences may even be underestimated because several parasites intermittently shed eggs or oocysts. Since no data was available on the infection status of the owners, no conclusions can be drawn about the risk for transmission of these pathogens. Transmission of parasites or protozoa to the human is only possible if the parasites have been developed into the infective stage, are pathogenic for humans (e.g. assemblage A for *G. duodenalis*), and ingestion of sufficient numbers of infective agents has taken place.

Acknowledgements

We thank the students Danielle Resink, Janne Teninga, Renée van de Ven and Kirsten Peeters for their help in collecting the materials in the veterinary practices. The study was funded in part by a research grant from the Foundation for the Dutch Cat Fancy and Foundation Kattenzorg, The Hague.

References


**G Model**

**VETPAR-4790; No of Pages 8**

P.A.M. Overgaauw et al. / Veterinary Parasitology xxx (2009) xxx–xxx


