Aspects of *Toxocara* epidemiology in the Netherlands

Epidemiologische aspecten van *Toxocara* in Nederland

(met een samenvatting in het Nederlands)

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Contents

Introduction
aim and scope of this thesis

Toxocara canis and *Toxocara cati* are intestinal helminths of, respectively, dogs and cats. Both *Toxocara* species have, because of their zoonotic significance, important public health consequences. Prevention of infection with *Toxocara* eggs is based on education (general public, veterinary practitioners and physicians), hygiene and deworming of pets.

The first two chapters are an overview of the literature of *Toxocara* infections in dogs and cats (***Chapter 1***) and human toxocarosis (***Chapter 2***).

Little is known about the prevalence of the infection in the different categories of dogs and cats in the Netherlands which is a prerequisite to give adequate information. This thesis describes surveys among privately owned dogs and cats and stray cats (***Chapter 3***), dog breeding kennels (***Chapter 4***) and catteries (***Chapter 5***) to determine patent infections in dogs and cats and environmental contamination with *Toxocara* eggs in breeding colonies as well as eventual risk factors for infection.

Activation of somatic *Toxocara* larvae followed by a tracheal migration and the development of a patent infection is suggested for cyclic bitches during metoestrus. To get a clear answer, a group of intact beagles was monitored over a two year period by regular faecal examination and determination of serum *Toxocara* titers during the period following each oestrus which was compared with similar observations in pregnant bitches (***Chapter 6***).

Deworming is considered as an important tool in the treatment of patent nematode infections and the prevention of environmental contamination with *Toxocara* eggs. The anthelmintic efficacy of oxibendazole against intestinal nematodes of dogs and cats is not reported conforming to the dosage and schemes used in Europe so far. A field study among dogs, cats and puppy litters was therefore performed with emphasis on the suppression of egg shedding by young animals (***Chapter 7***).

The knowledge of *Toxocara* epidemiology by veterinary practitioners, physicians, pet owners and non-pet owners is investigated to get a better understanding of the current practices of

education and the need for specific information (***Chapter 8***). The effect of a government education campaign performed in 1993 on awareness of *Toxocara* and toxocarosis on these groups is involved.

Finally the results are discussed in the context of data from the literature (***Chapter 9***).

Chapter 1

General introduction
Aspects of *Toxocara* epidemiology,
Toxocarosis in dogs and cats

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Introduction

Toxocara canis and *Toxocara cati*, roundworms of dogs and cats, are probably the most common gastrointestinal helminths of domestic canids and felids world-wide (81). The reported infection rates in Western Europe vary from 3.5% to 17% for *T. canis* in dogs and 8% to 76% for *T. cati* in cats. In the USA the figures are between 2% - 79% and 10% - 85% respectively (1, 25, 31, 79). The prevalence of patent *Toxocara* infections is highest in young dogs and cats and much less common in adult animals. *Toxocara* infection is the (covert) infection following ingestion of *Toxocara* eggs, or ingestion of larvae that can lead to (overt) clinical disease, which is presently called toxocarosis. The former name for toxocarosis was toxocariasis.

A good understanding of the epidemiology is required so that effective prevention of infection in man, dogs and cats is possible. In this review, the mode of transmission, clinical symptoms, diagnosis, prevention and control of toxocarosis in dogs and cats will be discussed, together with a critical appraisal of some of the less well-known issues.

Mode of transmission

Infectious agents

The life cycles of *T. canis* and *T. cati* are complex. Adult worms in the intestinal tract of infected dogs and cats shed large numbers of eggs via the faeces into the environment where they are ingested by natural hosts as well as paratenic hosts (Figure 1). In the intestine the larvae hatch and migrate via blood vessels all over the body. This is called visceral larva migrans (VLM). In young animals a tracheal migration occurs via the lungs and trachea and after swallowing, the larvae mature in the intestinal tract. In paratenic hosts and most adult dogs and cats that have some degree of acquired immunity, the larvae undergo somatic migration to remain as somatic larvae in the tissues. After predation of *Toxocara* infected paratenic hosts by dogs or cats, larvae will be released and develop in most cases directly to adult worms in the intestinal tract.

In the pregnant bitch and queen, ‘dormant’ tissue larvae are reactivated and migrate in the bitch across the placenta to infect the foetuses. New-born puppies and kittens also acquire infection through ingestion of larvae in the milk. (81, 96, 97).

Contamination of the environment with *Toxocara* eggs

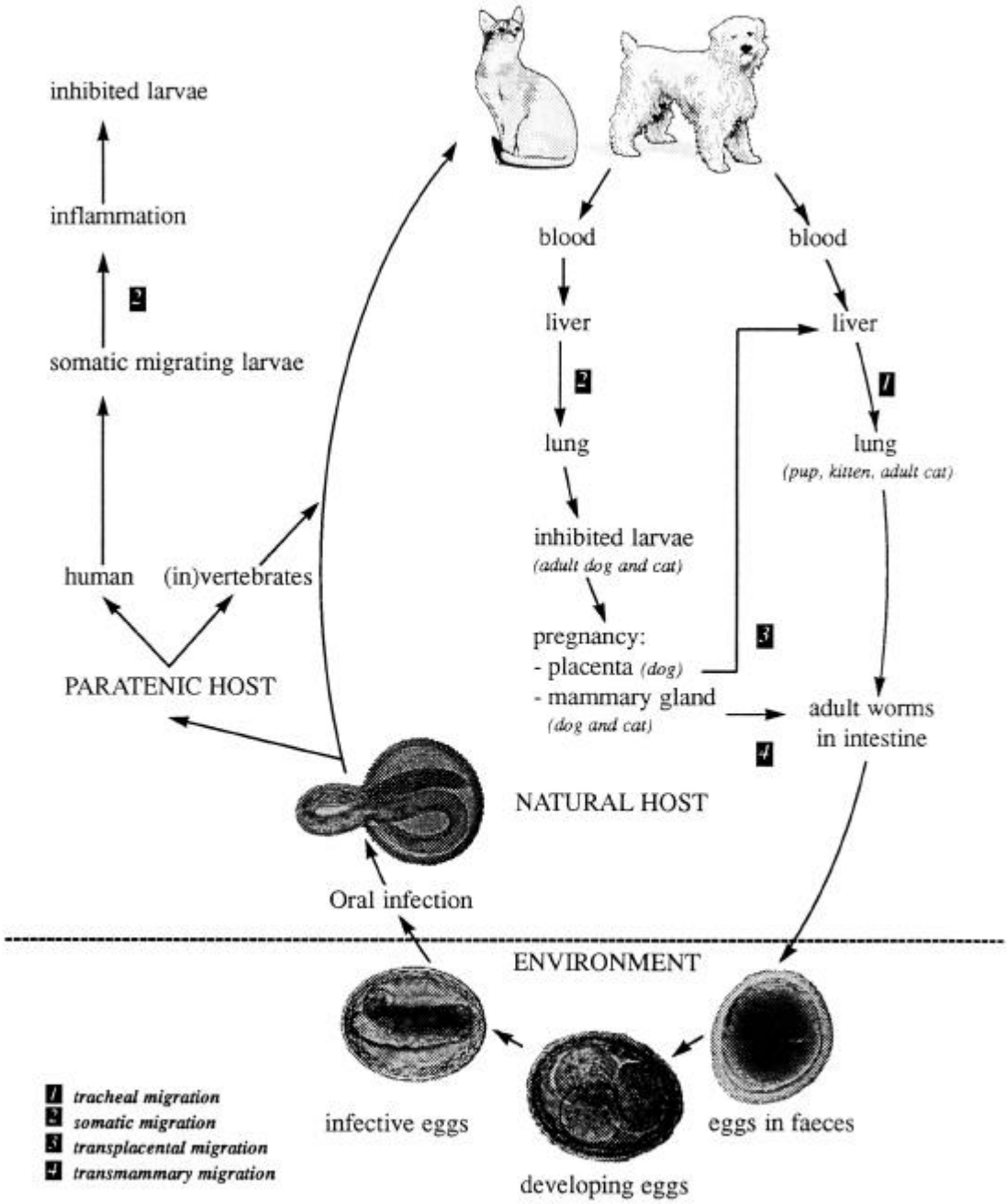
Toxocara eggs are unembryonated and not infectious when passed in the faeces of dogs and cats into the environment. Within a period of between 3 - 6 weeks to several months, depending on soil type and climatic conditions such as temperature and humidity, eggs will develop to an infectious stage that can survive under optimal circumstances for at least one year. No larval development occurs at temperatures below 10° C and larvae die below temperatures of –15° C (81). The 2nd larval stage or somatic larvae in the tissues has always been considered to be the infective stage; it has become clear that the infective stage is in fact the 3rd stage, reached after two moults in the eggshell (56).

Several studies from all over the world demonstrated high rates (10% - 30%) of soil contamination with *Toxocara* eggs in parks, playgrounds, sandpits and other public places (34).

Differentiation between eggs of *T. canis* and *T. cati* has not often been attempted (43, 71, 84, 105), but *T. canis* eggs are reported to be more commonly found in soil (35). Scanning electron microscopy (106) or microscopic differentiation of the fine pitting of the superficial layer of *T. cati* eggs compared to *T. canis* eggs (80) can be used to identify individual eggs reliably.

The source of the investigated soil, however, will certainly influence these findings. In a survey in the Netherlands, the presence of *T. canis* eggs in public parks was comparable with reports from other European cities, but most of the investigated sand-boxes were polluted with *T. cati* eggs. This was explained by the fact that cats prefer a quiet place with sandy material to defecate, while dogs only defecate in such places if owners force or educate them to do this (54). The same conclusion was drawn in a study where the defecation habits of cats were observed. Only 11 (1%) of the total 972 defecating animals in 3 sandpits during a 4.5 months observation period were dogs, the remainder were cats. Almost all of the cats were stray cats and between 25% (1/4) and 67% (8/12) of the cats that defecated in the different sandpits were infected with *T. cati*. Eighty per cent of the defecations occurred at night (106).

Figure 1. Life cycle of *Toxocara canis* and *Toxocara cati*.



Infection with Toxocara eggs: 1. Oral infection. 2. Tracheal migration. 3. Somatic migration.
Infection with Toxocara larvae: 4. Transplacental infection. 5. Transmammary transmission. 6. Through paratenic hosts.

Infection of dogs and cats with *Toxocara* eggs

Tracheal migration

After ingestion of infective *Toxocara* eggs by young dogs, a tracheal migration of larvae occurs through the liver, the vascular system and the lungs. The larvae break out into the alveoli and migrate to the trachea and pharynx. After swallowing, they complete their development in the stomach and small intestine. Eggs first appear in the faeces 4 to 5 weeks post-infection (22, 23, 35, 81). In a survey among dogs and cats in the USA (60), the highest prevalence of patent *Toxocara* infections was found in 2-week to 2-month old dogs and in 2- to 6 month-old cats.

Age resistance

By the time a puppy has reached the age of one to two months, the probability that newly hatched *T. canis* larvae will develop into adult ascarids falls to a very low level, while the probability of somatic migration progressively increases (38). The failure to produce patent infections in older dogs is termed age resistance and is not 'all or nothing' in nature, but rather a gradual decrease in the recovery rate of adult ascarids as the age of the dog advances (76).

A significant contribution of acquired immunity to this phenomenon has been exclusively demonstrated in several studies (7). The mechanism of resistance in mature dogs may operate partly within the lungs, perhaps as a delayed-type hypersensitivity response (23, 36). The difficulty in development experienced by the infective stage larvae to the next stage suggests that the resistance is directed against the infective stage of the parasite (76).

Development of immunity was shown in mice infected with embryonated *T. canis* eggs and described as immunosuppression after one *T. canis* infection, whereas two or three infections seemed to cause immunoprotection (19). To determine the origin of the

immune response, parasite naïve mice were injected with lymph cells, serum or both from mice infected with *T. canis* on days 1 and 100. Control mice received similar material but from parasite naòve mice. Comparison of the larvae after experimental infection revealed a significant reduction in the number of larvae in liver and lungs after cell transfer, whereas serum transfer decreased the number of parasites in brain and carcass. The combination of serum and cells showed a synergistic action in lungs and brain but an antagonistic activity in liver and carcass (7).

Löwenstein (66) infected one group of helminth-free bitches with infective *T. canis* eggs on the day of conception, followed by fenbendazole treatment (100 mg/kg) daily from day 30

until parturition to remove somatic larvae. A further challenge with infective eggs was performed during lactation. Control dogs received only the infective eggs during lactation. It was found that previous exposure to infection resulted in significantly fewer larvae being shed in the milk and fewer migrating to organs and tissues compared with control animals. Re-infection resulted in diarrhoea in all sensitised bitches; this suggested an allergic inflammatory reaction, tending to limit larval infection.

Similar results were reported from twice-infected mice showing a 27% reduction in the total larval recovery rate compared with non-sensitised controls. A significant number of larvae were retained in the liver and less larvae were recovered from the brain. An inflammatory reaction was seen in the gut wall of sensitised mice but not in the controls, again suggesting a role played by the intestine in the development of resistance to *T. canis* infection (3).

The reason why the host's immunity does not eliminate all tissue parasites is not understood; two mechanisms of evasion of the host's immunity by the larval stages have been suggested. One is hypobiosis of the tissue larvae that presumably reduces the production of protection-inducing antigens, thus rendering the parasite less susceptible to interference from the metabolism of the host. The other is immunosuppression by interference with the function of T-helper cells which inhibits the response to protective parasite antigens and the production of specific antibodies to these antigens (7).

Large and sustained doses of corticosteroids were able to break the resistance of a 6-month-old dog and allow development of patent infection (62). This increased susceptibility to infection was associated with decreased lymphocyte responsiveness in vitro to phytomitogens and *T. canis* antigens and suppression of eosinophilia in a study on periparturient bitches (64). The authors formulated the hypothesis that the immunosuppressive effect of pregnancy and lactation may permit tissue larvae, or larvae from a newly acquired infection, to undergo tracheal migration and subsequent intestinal development. In general, new infections of the lactating bitch will occur also by ingestion of immature fourth-stage larvae from vomit or faeces from the puppies. Larvae can develop to adults without a tracheal migration; this could also be the explanation for finding egg-producing *Toxocara* worms in the intestine. The finding of *Toxocara* eggs in the faeces of a bitch one week after parturition and prior to the detection of eggs in the faeces of her puppies leads to the hypothesis of tracheal migration of activated somatic larvae in the bitch. However, this bitch was experimentally infected during pregnancy with 10,000 eggs, representing a very high dose of infective eggs which may have caused immunosuppression or simply overcome the resistance.

Scothorn (94) found no evidence of intestinal infection with *T. canis* on necropsy examination of bitches at different times during gestation.

Another group that is reported to be at higher risk of *Toxocara* infection is the bitch during metoestrus. Three times as many bitches in this phase of the Oestrous cycle had patent *T. canis* infections as those in other groups (27). This comprised a pilot survey of 181 dogs during routine visits to a veterinary practice, but nothing was mentioned about the history of the dogs. The author assumed that prolactin is the major triggering factor for stimulation of somatic larvae. In addition to this, prolactin suppresses the immune response which enables the acquisition of new infections (28). Similar evidence supporting this hypothesis were not found in the literature.

Patent infection of adult dogs

Although the prevalence of *T. canis* is highest in young dogs, a certain proportion of the adult canine population can also be infected (72, 79, 88, 97, 104). Adult *Toxocara* worms may occur as a result of suppressed immune response, ingestion of low numbers of infective eggs (23) or following ingestion of infected paratenic hosts (110). Fully susceptible adult dogs have also been described, despite repeated egg exposure and development of antibodies (68). This could be related to certain breeds, e.g. Greyhounds in this particular survey, and the size of the dose of *T. canis* eggs. Since natural exposure of the canine population to infective *T. canis* eggs is more likely to be similar to the low-dose levels described by Maizels and Meghji (68), it was concluded that it may well be that the overall susceptibility of dogs developing a patent *Toxocara* infection is at a higher level than is currently accepted.

Toxocara infection and gender

Patent *T. canis* infection tends to be found more often in adult male dogs than female dogs (18, 33, 47, 48, 49, 68). A suggested explanation for this phenomenon is that female dogs harbour mainly resting larval stages in their somatic tissues which infect their offspring in the future, while continuation of *Toxocara* infection via male dogs can only take place by the spread of eggs from adult worms in their intestinal tract. The remaining, albeit theoretical survival route of somatic larvae would be if male dogs were themselves predated thus acting as paratenic hosts. Nothing is published, however, regarding significantly different findings on somatic tissue larvae between female and male dogs to give substantive support to this hypothesis.

In one survey *T. canis* was more frequently found in females than males (73). This was explained by the assumption that females were exposed to *T. canis* larvae passed out by their puppies.

Castrated males and spayed females had a lower prevalence of infection, compared to their respective entire counterparts, probably because they are less likely to roam and more likely to be better cared for (60, 109).

Tracheal migration in the cat

Following a tracheal migration after oral infection with infective *T. cati* eggs, adult worms are present by day 28 and eggs can be first observed in the faeces 56 days after infection (96). In contrast to the dog, the tracheal migration following *T. cati* egg infection in cats remains high even in older cats (72, 81) but less frequent than in young cats (72, 109). In neutered cats a significant decrease in prevalence of infection was found, compared to their respective entire counterparts (109).

Somatic migration

Adult dogs and cats can be infected by ingestion of infective *Toxocara* eggs from the environment, mainly contaminated soil. Larvae will hatch in the intestine and invade the mucosal layer. Migration occurs either passively via lymph and blood or actively by penetration of the tissues and invasion of all parts of the body. Gradually somatic larvae accumulate in the tissues (somatic migration), persisting for long periods in a manner similar to that seen in paratenic hosts (97). Larvae of *T. cati* prefer to migrate to the muscles (93), while *T. canis* larvae were more found in the central nervous system (97). In mice, it was found that more than half of the embryonated eggs appeared to be discharged without hatching in the intestine. The emptying time of the gastrointestinal tract appeared to be one of the important factors. If eggs remained longer in the intestine, e.g. with a full stomach, 15 to 20 per cent more eggs hatched (74).

Infection of dogs and cats with *Toxocara* larvae

Transplacental migration

Several studies have shown that nearly 100% of puppies are infected in utero from day 42 of the gestation by somatic larvae (63, 94). This so called transplacental migration or

intra-uterine infection is the most important mode of transmission in dogs; 98.5% of activated larvae is reported (16). In cats, prenatal infection via the placenta does not occur (96, 103).

The larvae in pregnant bitches are reactivated by one or more unknown factors; the changing hormonal status of the bitch during pregnancy has been suggested. Oshima (75) found that injections of the gonadotrophin prolactin led to a marked fall in the number of larvae in tissues of mice that were experimentally infected with *T. canis* and suggested that this hormone is involved in stimulating dormant larvae to resume their migration. Within hours of birth, the larvae that were present in the liver of the neonate, migrate to the lungs and undergo a tracheal migration. Adult worms can be found at two weeks of age (64) and large numbers of eggs may be passed in the faeces after a minimum period of 16 days (65, 88).

Little is known about the numbers of larvae which can be found in the tissues of the bitch, the proportion of tissue larvae activated in pregnancy and the survival time of larvae in the tissues (65). Transmission occurs in consecutive pregnancies, even in the absence of re-infection between each parturition (15).

Transmammary transmission

After activation somatic *Toxocara* larvae of dogs and cats will also be transmitted via the colostrum and the milk (transmammary transmission, lactogenic- or milk-borne infection). Following ingestion by the offspring, the larvae undergo development without tracheal migration. Larvae are found to pass in the bitch's milk for at least 38 days after parturition (111).

This route is less important than intra-uterine transmission in the puppy, but it is the primary mode of infection in the kitten (103). In a study in puppies, only 1.5% of *T. canis* larvae were transmitted via the lactogenic route (16).

Kittens infected by lactogenic transmission will show faecal egg excretion 9 days earlier than in egg infected cats (by 47 and 56 days after infection respectively). After ingestion of an infected paratenic host this period is comparable with the period after transmammary infection (96).

Infection of the dam by the offspring

A nursing bitch or queen may acquire a patent *Toxocara* infection by ingesting intestinal larvae expelled in vomit or faeces of their offspring during nursing. A number of these larvae mature and become patent (98). Together with the ingested eggs from the faeces of their offspring, lactating dogs and cats can in this way disseminate large numbers of eggs into the

environment. Between 4 to 10 weeks after parturition, these infections will disappear spontaneously (64, 88).

Oshima (75) suggested that the suppression of resistance to infection during lactation and the subsequent development of *T. canis* in the intestine is explained by the special influence of lactation and the relationship with the secretion of prolactin. This seems to be confirmed by the finding that such infections are eliminated spontaneously within one week following the cessation of lactation (64).

Finally, *Toxocara* eggs shed in the faeces of puppies or kittens can be ingested by the mother, where they pass through the digestive tract, causing a false-positive diagnosis of *Toxocara* infection upon faecal examination.

Transmission through paratenic hosts

Paratenesis is the mode of infection of some larval nematodes like *Toxocara*, ensuring its continuing survival by its distribution in prey species (39).

This route of infection exists because of the development of somatic larvae in paratenic hosts, including vertebrates such as rodents and birds or invertebrates such as earthworms and insects (e.g. flies). Small mammals are suggested to play an important role as paratenic hosts in urban and rural localities. In the Slovak Republic, 22% of the investigated small mammals were found seropositive for *Toxocara*, most frequently in several species of mice (24).

A small fraction of the larvae in mice were found to migrate from the lungs to the intestine, following a tracheal migration. This resulted in an unexplained subsequent re-invasion of tissues by larvae after entering the intestine for the second time 5 days after infection (74).

After ingestion of a paratenic host infected with *T. canis* larvae by the dog (97) or *T. cati* larvae by the cat (81, 96, 103), the larvae develop directly in the intestine because the larvae have already migrated in the preceding host, and presumably have reached an appropriate stage of maturity such that they can develop into adults in the intestine. In contrast with these conclusions, Warren (110) reported that *T. canis* larvae undergo a tracheal migration to adults by 19 days in dogs following their ingestion via an experimental paratenic host (mice tissues). The difference between the two experiments however, was the time of infection of the mice which were fed to the dogs. Infection of these mice in Sprent's study (97) was two to six weeks before, whereas they were infected only four days before being fed to the dogs in Warren's experiment. It can be assumed that four days is too short for developing the stage of maturity that is responsible for direct development in the intestine of the dog after ingestion.

Publishing different conclusions of similar experiments without giving possible reasons such as the time of infection of the fed mice can create confusion.

The paratenic route seems to play a significant role in certain wildlife populations like foxes (81) and might be of importance for hounds (8). In cats infected with *T. canis*, disseminated eosinophilic and granulomatous disease with marked pulmonary artery and airway lesions as a visceral larva migrans (VLM) like syndrome was seen (83).

Clinical symptoms, haematological findings and pathology

The clinical symptoms depend on the age of the animal and on the number, location and stage of development of the worms. *Toxocara* infection is highest in puppies (94, 108) and kittens up to 6 months of age (96, 109).

Dogs

Puppies

Prenatal infection of puppies is suggested to be responsible for stillbirths and early deaths (94), but this has never been reported in other studies. After birth, puppies can suffer from pneumonia associated with the tracheal migration and die within 2 to 3 days. At an age of 2 to 3 weeks, puppies can show emaciation and digestive disturbances, caused by mature worms in the stomach and intestine. Diarrhoea, constipation, vomiting, coughing and nasal discharge can be found at clinical examination. Distension of the abdomen ('potbelly') can occur, probably as result of gas formation caused by dysbacteriosis. Mortality is possible due to obstruction of the gall bladder, bile duct, pancreatic duct and rupture of the intestine (81). After superinfection with *Toxocara* the eosinophil count is raised with a peak on the 8th day that may last for more than 50 days. The average peak level was indicated as high (0.5 - 1 x 10^9), but this falls between the limits (0.1 - 1.25 x 10^9) and cannot be considered as an eosinophilia. The rise in eosinophils was suggested to be indicative of an allergic reaction by the host (76).

Adult dogs

A low infection rate of patent *T. canis* infections of adult dogs is the reason that clinical symptoms are rare. During somatic larval migration, dogs seldom manifest signs of clinical

disease (9). Migrating larvae induce high levels of liver enzymes (AST, ALT) with a peak 3 days after infection with embryonated eggs. The total IgG levels in serum double during 20 days post infection and lymphocyte responses to T-cell (phytohem agglutinine, PHA) and T and B cell (pokeweed mitogen, PWM) mitogens have been markedly suppressed for at least 10 days (99). The larval titre, established by the ELISA test using TES (*Toxocara* excretory-secretory) antigens, and the number of eosinophils increase (111). Larval migration to the eyes (OLM) of *T. canis* larvae is described (45, 55).

Cats

Kittens

The situation in kittens is different from puppies, because primary infection is only by transmammary infection. Kittens are older when worms are maturing (adult from day 28 and egg producing from day 49 after birth); tracheal migration with related symptoms is not present. Therefore, kittens have a better chance to grow and in the meantime develop better bodily condition before problems may be seen. For this reason clinical symptoms similar to those in puppies are usually inapparent and occur at an older age than in puppies.

Adult cats

The cat with more severe intestinal infections can show a potbelly, rough coat and signs of dehydration in cases of diarrhoea (42). Parsons et al. (82) described a disseminated granulomatous disease caused by migrating *T. canis* larvae in a cat; this can be assumed to be comparable with a *T. cati* infection.

Diagnosis

Patent infections

Patent *Toxocara* infection in dogs and cats can be tentatively diagnosed from the medical history, particularly the use or otherwise of an appropriate anthelmintic schedule, and the clinical symptoms. Confirmation of the diagnosis can be obtained by finding dark brown coloured eggs with thick pitted shells in faecal samples. The direct faecal smear technique is not a sensitive test and generally should never be used for recovering eggs from faecal samples (61). Examination of faeces by a floatation technique is a useful method for detecting helminths (42). The specific gravity of floatation solution should be at least 1.18 and

centrifugation is preferred (22). The specifications of the floatation test for finding *Toxocara* eggs were calculated as 51% for the specificity, a sensitivity of 100%. The predictable value of a positive test is 100% and 81% for a negative test (79).

Non patent infection

The ELISA test, using TES antigens, is described as a sensitive technique for determining whether or not a bitch is carrying somatic larvae (91).

Methods of control

Preventive measures

There are two reasons for *Toxocara* control; to prevent human infection and to reduce the risk of infection to pets.

Toxocara eggs are very resistant to adverse environmental conditions and remain infective for years. Since no practical methods exist for reducing environmental egg burdens, prevention of initial contamination of the environment is the most important tool. This can be achieved by taking measures such as eliminating patent infections in dogs and cats, preventing defecation by pets in public areas, hygiene, and education of the public (77, 37).

Prevention of contamination of the environment

High degrees of environmental contamination can be expected in places where dogs and cats are concentrated such as training schools, animal shelters and breeding kennels (49). No correlation could be established between pet ownership and the presence of *Toxocara* spp. eggs in suburban gardens (84). Household garden soil was found to be a potentially greater source of *Toxocara* infection than soil in public green areas (43).

A decrease in contamination can be achieved by methods including: restriction of uncontrolled dogs and cats, cleaning up faeces from soil and on pavements by dog owners, preventing access of dogs and cats to public places (especially children's playgrounds) and by use of strategic anthelmintic treatment of dogs and cats with emphasis on puppies, kittens, nursing bitches and queens (58, 90). T*oxocara* eggs are not destroyed by composting and can survive sewage treatment (65).

A complicating factor in the prevention of environmental contamination is the presence of infected wild and stray canines and felines. In Europe the wild fox is nowadays more common in urban areas and stray cats are familiar in every neighbourhood (87). In surveys in the

Netherlands, foxes (12) and stray cats (79) were found to be heavily infected with *Toxocara* (74% *T. canis* and 21% *T. cati* respectively). Stray dogs in Switzerland were found to be infected with *Toxocara* in 17% of cases (21). A high prevalence (75%) of stray dogs infected with *T. canis* is reported from the Slovac Republic (24).

Anthelmintic treatment strategy

The most serious and concentrated source of infection is the bitch nursing a litter and puppies aged between 3 weeks and 6 months (49). A major aim of long-term prophylactic treatment programmes is to suppress *T. canis* egg-output throughout the whole of puppyhood using a multidose schedule (51). Anthelmintic treatment should be started before the age of 3 weeks. Because milk transmission occurs continuously for at least 5 weeks post partum, repeated treatments are necessary. Larvae that reach the intestine need at least 2 weeks to mature and start passing eggs, therefore the treatment should be repeated every 14 days.

Re-infection can occur throughout the suckling period (30) and treatment should at least be continued until the time when the last larvae arrive through the milk in the puppies' intestine at 7 weeks of age (8). Bitches should always be included in the treatment at the same time as the puppies.

Advice regarding the initial anthelmintic dose for kittens can cause confusion, because prenatal infection does not occur in kittens and egg excretion begins later than in puppies (96). Therefore, preventive treatment in kittens can usually be instituted effectively at 6 weeks of age.

Control in older dogs and cats can be achieved by periodic treatments with anthelmintics whose efficacy can be limited to the intestinal stages, or by treatments prescribed based on the results of periodic diagnostic faecal examinations (5).

In the Netherlands the following schedule to prevent egg output is advised: puppies should be dewormed every two weeks from 2 to 8 weeks of age and kittens from 4 to 8 weeks of age, followed by treatments every 2 months until 6 months of age. Nursing bitches and queens should be treated concurrently with their puppies and kittens respectively and every other dog and cat twice a year. This advice corresponds closely with the 'Recommendations for Veterinarians' of the American Association of Veterinary Parasitologists in 1994. Similar programmes were previously described in 1978 (58, 85).

Efficacy of anthelmintics against the adult stage of Toxocara

Lower efficacy values of anthelmintics were found in puppies and kittens suffering diarrhoea which is often caused by the stresses of weaning, change of diet and moving to a new environment.

The rate of passage of ingesta through such very small animals may influence the efficacy of the anthelmintic and therefore repeat dosing is advised as a single dose may not always give the desired effect (51, 67, 102).

Even daily treatment of young dogs, in combination with weekly application of infective *T. canis* eggs to simulate natural continuing exposure, could not prevent egg output in all animals (69). Monthly (ivermectin, milbemycin) or daily administration (DEC/oxibendazole) of anthelmintics in lower dosage in a heartworm prevention schedule were found to provide optimal suppression of *T. canis* egg counts (86).

Despite voluntary anthelmintic treatment of dogs by owners, patent infections of varying incidence are found in every survey among dogs and cats; it can be concluded that this has only a limited practical efficacy in the control of toxocarosis in dogs, cats and humans (7). Experimental treatment of patent *Toxocara* infections in dogs revealed high efficacy rates (17, 51), but the same treatment in practice often showed lower efficacy. It is not known whether the treatment itself is inefficacious or re-infections are more frequent (8). Anthelmintic resistance of *Toxocara* has not been reported to date, in contrast to some parasites in ruminants and horses (20).

More trials with anthelmintics under field conditions are therefore required to provide a comparison with experimental results, although it would be difficult to find a reliable source of animals with patent infections. Puppies can be used more easily for this purpose since all are born infected with *T. canis*; much more still needs to be elaborated regarding the efficacy of anthelmintics in unweaned puppies (46). Guidelines have been designed for studies for the assessment of the efficacy of drugs against helminth parasites of dogs and cats (52).

Efficacy of anthelmintics against somatic larvae

Elimination of the larvae from the tissues and therefore prevention of vertical intrauterine and transmammary transmission would have a significant effect on the parasite population (56). Deworming of bitches during pregnancy is sometimes advised in anthelmintic schedules (40), but this advice is questionable. Efficacy of anthelmintics against somatic larvae in experimental animals (mice) and bitches (not in queens) has been intensively investigated.

In a report about the efficacy of anthelmintics in experimentally infected mice (2), it was mentioned that small laboratory animals present difficulties when used for screening of

anthelmintics targeted at domestic animals and human beings, e.g. a certain disproportion in the dose volume related to the digestive tract. In other aspects such as monitoring larval migration, studying histopathological changes and enumerating larvae in the body, small laboratory animals have obvious advantages.

Nicholas and Stewart (70) showed that a four week treatment of mice infected with *T. canis* with fenbendazole at a dose of 20-30 mg/kg per day (starting 10 days after infection) killed almost all larvae, but the same dose over two weeks did not. Furthermore, neither oxfendazole nor fenbendazole were effective against larvae 8 to 12 weeks after infection.

Holt et al. (44) did not demonstrate any significant reduction in larvae in the brains of infected mice when treated for 4 days with high doses of 6 different anthelmintics (piperazine 4 g/kg, mebendazole 0.15-0.3 g/kg, oxfendazole 2 g/kg, albendazole 1 g/kg, fenbendazole 2.5 g/kg and diethylcarbamazine 0.2 g/kg) starting directly after infection or at 19 days later.

To investigate if the blood-brain barrier may be preventing anthelmintics from reaching the CNS in effective concentrations, mice were treated with fenbendazole and flubendazole at various intervals after experimental infection. The larvae in brain and muscles were equally vulnerable to the drugs and it was concluded that the brain of mice does not seem to provide a site facilitating survival of larvae during anthelmintic treatment (56).

A deworming schedule of the pregnant bitch with fenbendazole (50 mg/kg/day), starting at the 40th day of pregnancy until the 14th day postpartum resulted in an 89% decrease of *T. canis* in puppies (15, 59). If the treatment schedule was stopped on the day of parturition, only a 64% reduction of *T. canis* could be established (15).

Bosse and Stoye (13) obtained larvae-free puppies when bitches, experimentally infected with 20.000 *T. canis* at D0 of pregnancy, were treated with albendazole, fenbendazole and oxfendazole at a dose of 100 mg/kg per day from the 30th day of pregnancy until parturition.

Fenbendazole or albendazole at a dose of 150 mg/kg/day for 3 days, showed a reduction of somatic larvae from the tissues other than the brain (see also Holt, 1981) of non-pregnant bitches by more than 95% (64). The dogs in this trial were between 7 to 9 months of age, treated 5 weeks after experimental infection with *T. canis,* and examined at post mortem 4 weeks after treatment. It was concluded that administration of these benzimidazoles before pregnancy might be helpful in the prevention of prenatal transmission of *T. canis* to future litters, but it will not prevent transmission of infection if the bitch acquires a new infection during pregnancy or early lactation. This finding is remarkable and in contrast with the opinion that only larvae activated during pregnancy are susceptible to the action of anthelmintic drugs, particularly during migration from the granuloma (56, 81). As in several

other studies, the dogs were treated at a stage relatively early after infection which cannot be considered to be comparable with naturally infected dogs.

The influence of the time of experimental infection in relation to the efficacy of the treatment was also indicated by the results from a three day treatment of mice with benzimidazoles at a dose of 100 mg/kg per day (101). Treatment at 90 days after experimental infection showed a reduction of one third of the larvae, while at D120 no significant reduction could be established.

Early (at D25 and D35) and late (at D45 and D55) treatment of pregnant bitches, also experimentally infected at D0 with *T. canis* eggs, with 0.3 – 1.0 mg/kg ivermectin (not approved for this use) resulted in 34% and 94% reduction of adult *Toxocara* worms in the intestine of the puppies respectively (59). In both treatment groups the egg output was still high in the offspring. In another study bitches, experimentally infected at D0 of pregnancy, were treated with 2 injections of doramectin (not approved for this use) at a dose of 1 mg/kg at D40 and D55 of pregnancy. The treatment did not eliminate somatic larvae in the bitch and did not completely prevent perinatal infections with *T. canis* (92). In all puppies egg output started at D28 after birth, but the puppies from treated bitches had lower egg counts. Four of the 5 treated litters and the control litter showed intestinal stages post mortem.

Only complete prevention of intra-uterine and lactogenic infections can be regarded as effective, because even puppies with a low infection rate will contribute considerably to the contamination of their environment with *T. canis* eggs (92). Anthelmintics at the recommended doses are not effective against inhibited somatic larvae (26) and treatment of bitches before mating and two weeks before the anticipated whelping date has no useful effect on prenatal transmission (30). Therefore it is not advised to deworm pregnant dogs and cats, but repeat dosing during puppyhood is a necessity (10, 30, 64). Furthermore, long and precise treatment schedules during pregnancy are not compatible with practical use because of considerations such as expense (7) and the risk of toxic side effects on the foetuses. After treatment of bitches during pregnancy (100 mg/kg albendazole, fenbendazole and oxfendazole from D30 of the pregnancy until birth) low birth rates and palatoschisis (albendazole and oxfendazole) were reported (13).

In relation to the reported efficacy of anthelmintics against somatic larvae, an important restriction should be considered. Swerczek (1971) investigated the transmammary passage of *T. cati* in the cat and found a marked difference between the yield of larvae from tissues in queens killed with sodium pentobarbital (low yield of nonviable larvae) and ether (high yield and viability). He concluded that sodium pentobarbital or similar drugs kill or immobilise the

larvae and should not be used when Baermannized tissues are examined quantitatively for larvae. In studies, published after 1971, in which larvae were recovered to determine influences on somatic larvae, the way of euthanasia of the experimental animals is seldom described (2) and in the majority of the reports omitted (13, 64, 70, 92, 101). This can therefore be ultimately a confounding factor for the results.

Immunisation

Any method of effective control should destroy *T. canis* in the reservoir provided by the tissues of adult hosts. As described before, the dormant hypobiotic larvae are highly resistant to anthelmintics. Therefore an anti-parasitic activity is required that can destroy activated *T. canis* larvae as well as newly acquired infections. Ongoing administration of anthelmintics is neither a practical nor a reliable solution. Immunisation on the other hand, is inherently long-lasting and more potent (7).

Immunisation of mice with *Toxocara* larval extracts or metabolic excretion/secretion products showed a certain level of immunoprotection and it was concluded by Barriga (7) that an effective anti-*T. canis* vaccine could be expected in the future.

Vaccination of mice using ultraviolet irradiated embryonated *T. canis* eggs showed the best protection after re-infection, while ES-antigen afforded less protection and whole adult worm vaccine and whole L2 culture vaccine gave no protection (3).

Oral or parenteral immunisation of mice on three or more occasions with *T. vitulorum* eggs induced significant protection against challenge doses of eggs. The most effective were three or more injections with peri-enteric fluids from adult worms, inducing 100% protection (4). Differences in the immunogenicity of the parasite species might account for differences between results as well as the immunisation regimes and adjuvants used.

Hygiene.

For dogs and cats hygiene can be achieved by removing the faeces (89, 100) and thorough cleaning of kennels (95). *Toxocara* eggs are highly resistant to a wide range of chemicals, but are killed by ultraviolet light, aqueous iodine and high temperatures. It is advisable to start cleaning kennels with a 20% solution of commercial bleach (1% sodium hypochlorite). This does not kill the eggs, but removes their sticky outer protein coat. These decorticated eggs are then easier to remove from inaccessible areas (32, 81). On the other hand, the infectivity of eggs treated in this manner will rise significantly and higher recovery rates are found in infected animals compared with situations in which the outer layer is left intact (74). Finally,

expulsed worms should be destroyed and predation and scavenging on carcasses by dogs and cats should be prevented.

Education.

Dog and cat owners can help to avoid contamination of the environment with *Toxocara* eggs and the exposure of other persons to unnecessary risks of *Toxocara* infections. Proper information about this zoonosis and the social concept of responsible pet ownership is required. Pet owners should be advised about deworming schemes, effective anthelmintics and to prevent their animals from defecating on children's playgrounds (53).

The important role that the veterinarian can play in this education process was described in 1980, when the American Veterinary Medical Association adopted the following resolution concerning *Toxocara* infection: '*The AVMA recommends that veterinary practitioners conduct appropriate client information programs with the goal of reducing the prevalence of infection in pet dogs and minimising the potential of transmission to human beings'* (6). It is recommended that information disseminated to pet owners should include the following:

- A description of *T. canis* and *T. cati* and how they affect their hosts;
- A description of prenatal and transmammary transmission;
- An explanation of how *Toxocara* can be transmitted to and produce damage in humans;
- An explanation of how prevention can most effectively be achieved;
- Information on how pet owners should routinely collect and safely dispose of their pets' faeces, especially from children's play areas;
- Advise children not to play in potentially contaminated environments (5).

Although veterinarians should be the most appropriate sources of information for their clients regarding the dangers and the control of toxocarosis, several surveys (41, 57, 78) have demonstrated that client education on this issue is lacking.

Treatment of patients

When a patent *Toxocara* infection is diagnosed in dogs and cats, worms should be removed by treatment with an effective and larvicidal anthelmintic with a low toxicity. In the Netherlands, benzimidazoles (fenbendazole, flubendazole, mebendazole, oxfendazole and oxibendazole), probenzimidazoles (febantel), pyrantel, levamisole and (only for dogs) nitroscanate are registered for this indication. Benzimidazoles have in general significant larvicidal effects (2, 46) and are virtually without toxicity at therapeutic dose rates (11, 29, 107). Piperazine, nitroscanate, levamisole and pyrantel are not efficacious against immature

worms and should be used routinely in adult animals only (14, 29, 46, 67). Levamisole is fairly toxic, especially in cats and an injectable formulation should never be used (11). Most anthelmintics for dogs and cats are evaluated in adolescent or adult dogs (50), but the most important treatments are those administrated to suckling puppies and young kittens. Due to concomitant physiological changes and the complexity of the host-parasite relationship, it is important that anthelmintics intended for control of *T. canis* and *T. cati* should be evaluated by means mimicking as nearly as possible conditions of field usage (51). If the efficacy of anthelmintics is evaluated by post mortem worm counts after treatment, high efficacy values can be obtained even when less potent compounds are used. This can give a false sense of security as they provide no information about the ante mortem faecal egg-output (46). It can result in marked differences, e.g. in one study the worm burden had been reduced by 86.2% following treatment with piperazine, but the egg-output, when compared with that of untreated controls, was hardly suppressed at all (30). In addition to the population dynamics of *Toxocara* (somatic migration of larvae at the moment of deworming and re-infection throughout the suckling period), there are other considerations that must be taken into account which necessitate repeated administration after 10 to 14 days on at least one occasion in adult animals and several times in juveniles (46, 64, 81). These considerations include: the different intestinal physiology of the unweaned puppy, the influences on the efficacy of anthelmintics such as diarrhoea (increased intestinal passage) and the fat contents of the bitches' milk.

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Chapter 2

General introduction
Aspects of *Toxocara* epidemiology,
Human toxocarosis

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Introduction

Toxocara canis and *Toxocara cati*, roundworms of dogs and cats respectively, are zoonotic parasites that cause widespread and common human infection (80). *Toxocara* infection is the (covert) infection following ingestion of *Toxocara* eggs, or ingestion of larvae that can lead to (overt) clinical disease, which is presently called toxocarosis. The former name for toxocarosis was toxocariasis.

A good knowledge of the epidemiology is essential so that this important problem in humans may be effectively prevented. In this article, the life cycle and transmission of infection of *Toxocara*, clinical symptoms, diagnosis and methods of control of toxocarosis in humans will be discussed, together with a critical appraisal of some of the less well-known aspects of this disease.

Infectious agents

The life cycles of *Toxocara canis* and *Toxocara cati* are complex (see also Part I: '*Toxocarosis in dogs and cats'*). Dogs and cats with patent *Toxocara* infections shed large numbers of eggs in the faeces. In the environment these eggs become infective after an embryonation period of several weeks.

Natural hosts as well as paratenic hosts, including the human, can ingest infective eggs. In the intestine the hatched larvae invade the mucosa and migrate via blood- and lymph vessels all over the body. In young dogs and cats a tracheal migration occurs via the lungs and trachea and after swallowing, the larvae reach the intestine where they mature.

In paratenic hosts and in most adult dogs and cats that have some degree of acquired immunity, the larvae undergo somatic migration to remain as dormant larvae in the tissues. After predation of a paratenic host by dogs or cats, these larvae are released and develop into adult worms in the intestinal tract.

In the pregnant bitch and queen the tissue larvae are reactivated and migrate to the placenta and mammary gland. In the bitch the larvae migrate through the placenta to infect the foetuses. New-born puppies and kittens also acquire infection through ingestion of milk containing larvae.

The prevalence of patent *Toxocara* infections is highest in young dogs and cats and is much less common in adult animals (69, 89, 90).

Mode of transmission to the human

Toxocarosis is a public health problem. Man acts as an unnatural host in which *Toxocara* larvae will not develop but migrate and survive for a long time.

The mode of transmission to humans is by oral ingestion of infective *Toxocara* eggs from contaminated soil (sapro-zoonosis), from unwashed hands or consumption of raw vegetables (26, 29). Some infections may occur from ingestion of larvae in under-cooked organ and muscle tissue of infected paratenic hosts such as chickens, cattle and sheep (2, 63, 75, 92).

Transmission to the second generation is theoretically possible. In mice, prenatal infection with *T. canis* (4) and *T. cati* (81) could be proved after infection of mother mice with *Toxocara* during mid pregnancy, but not after reactivation of inhibited larvae. Lactogenic infection was found in mice after experimental infection at the beginning of the lactation but, in contrast to prenatal infection, also following reactivation of inhibited larvae of *T. canis* (4). Vertical transmission in man as result of activated somatic larvae similar to situation in the dog, was investigated by examining maternal and cord blood sera. Cord blood sera of females that tested positive for *Toxocara* were investigated for IgM anti-*Toxocara* antibodies. All sera tested negative and it was concluded that transplacental migration does not occur in the group studied (95).

Importance of *Toxocara cati*

The role of *T. cati* as a zoonotic parasite is not always clearly recognised. Second or third-stage larvae (42) have already been found in 1956 in various potential paratenic hosts like chickens, mice, dogs and lambs, after experimental infection with *T. cati* eggs (89). The large number of common antigenic fractions shared between *T. canis* and *T. cati* and the similarity in the mode of infection make it difficult to define the respective role of these two ascarids in the aetiology of VLM (70).

Despite the fact that differentiation between *T. canis* and *T. cati* infections is not performed in surveys, the majority of reported human cases of toxocarosis in the past have been associated with *T. canis* and not with *T. cati*. Other authors do not distinguish the zoonotic role of both roundworms (6, 22, 24, 40, 73, 101).

The preference for *T. canis* is supported by several arguments. Exposure to dogs, rather than cats with the fastidious defecation habits of the latter, should be an important factor favouring *T. canis* infection (12, 26, 103). The more aggressive behaviour of *T. canis* in paratenic hosts

(90), the habit of cats covering their faeces in soil tends to prevent the spread of eggs (38), give further support to this preference for *T. canis* infection. The greater abundance of *T. canis* eggs in park soil and playgrounds (26) might be considered to be another factor; however, this last argument can be questioned because it depends on the source of the investigated soil. In a survey in the Netherlands, the presence of *T. canis* eggs in public parks was comparable with reports from other European countries, but most of the investigated sand-boxes were polluted with *T. cati* eggs. Cats prefer a quiet place with sandy material to defecate, while dogs only defecate in such places if the owner forces or trains them to do this (39). The first argument concerning risk exposure to dogs was weakened in the same article (26) by the remark that direct contact with infected dogs or cats may be a less important source of infection, because the time required from embryonation of eggs to attainment of infectivity is at least two weeks.

An interesting finding that should support the opinion that *T. cati* is less important than *T. canis* was reported from a survey in Iceland (103). In this country, dogs have been prohibited since the forties to control hydatid disease, but there is a large population of cats. None of the 307 adult Icelanders tested were found to be positive for *Toxocara*. Nothing was mentioned however about the cat population density, prevalence of *T. cati* in cats, the rate of environmental contamination, the expected survival rate of eggs in Icelandic soil and the assumed risk of soil contact by Icelanders.

To get a better understanding of the role and importance of *T. cati*, differentiation of *Toxocara*-antibodies in sera of infected human is required. Monoclonal antibodies that can distinguish between *T. canis* and *T. cati* are described (7) and a specific Ouchterlony's diffusion-in-gel method using adult worm extracts permitted not only a reliable diagnosis of toxocarosis but also species differentiation (64). Recently, a very sensitive polymerase chain reaction was reported to be able to detect DNA of *T. canis* in liver tissues of experimentally infected mice (105). In this way arguments for the importance of *T. cati* as a zoonotic infection may finally be ratified with the result that more specific information for prevention of this disease will become available. Until now, such a clarifying, distinguishing survey has only been published for patients with ocular larva migrans syndrome (OLM) where *T. cati* was suggested to play an important role (71).

Direct contact with animals

As mentioned earlier, direct contact with infected dogs or cats is not considered as a potential risk because embryonation of *Toxocara* ova to the stage of infectivity requires a minimum of

2 weeks. An exception to this is provided by an increased infection risk by direct contact with the nursing bitch and her puppies if subsequent deworming is not performed. It has been suggested that the litter area and the puppies' coats can be contaminated with infective eggs (79). However, after being passed in the faeces, *T. canis* eggs need between 9 to 15 days under optimal moisture and temperature conditions (25 - 30º C), and 35 days at 16.5º C to develop into infective larvae (69). Therefore it is unlikely that these eggs will remain on the puppies' coats for all that time.

T. canis infections are more likely to be a hazard for people exposed to contaminated environments. This seemed to be confirmed in a survey by Woodruff (102) among 102 British dog breeders that showed a significantly higher degree of infection (15.7% ELISA positive) compared with 922 healthy adult controls (2.6% positive). In other studies in which animal hospital employees (24), kennel workers (37) and cat breeders (103) were involved, no serological evidence of an increased risk could be established. The suggested explanation was the reasonable standard of personal hygiene by the personnel.

Importance of other ascarids

Other ascarids besides the genus *Toxocara*, i.e. *Toxascaris* and *Baylisascaris* have been shown to be causative agents of rare cases of visceral larva migrans syndrome (7).

A visceral larva migrans (VLM) is described for *Toxascaris leonina* to musculature, liver and lungs in experimental animals like mice (60). Zoonotic implications of this parasite, however, are neither suggested nor excluded. Lactogenic transmission is found in mice as paratenic hosts (41).

More specific immunodiagnostic tests are therefore required to clarify this question.

Infection risk to children

Children were reported to be more frequently infected than adults and VLM with more severe clinical symptoms is mainly found in children of 1 - 3 years of age (26, 45, 80). This can be explained because young children play often and have closer contact with potentially contaminated soil in yards and sand-pits. In addition, children may often put their fingers into their mouth and sometimes eat dirt (26, 36, 104, 106). In contrast with the existence of clinical toxocarosis in mainly young children, anti-*Toxocara* titres are more frequently found in older people (46, 61). This was explained by the fact that *Toxocara* titres last for many years where the infection pressure can be assumed as constant. This prevalence is therefore

the result of incidence over a course of time, and the increasing prevalence with age indicates a relatively high incidence of *Toxocara* (re-infection).

The tendency of some children to eat dirt (geophagic pica) is a major risk factor for infection. The compulsion to eat dirt as a behaviour disorder may affect 2% to 10% of children between the ages of 1 to 6 years (80). Pica is often associated with iron or zinc deficiency (88). Around 40% of patients with ocular involvement showed a history of pica (77).

Relationship between the presence of (young) dogs in households and OLM (34, 77, 78, 104) or the presence of antibodies to *Toxocara* (27, 36) has been described. These studies do not provide a reliable indication that these dogs were the sources of these infections. This is because public green areas are also frequently contaminated with infective eggs and therefore a significant association could be established between *Toxocara* infection and pica of faeces, soil or grass (27). Other surveys did not support a relationship with exposure to dogs or cats in the household (8, 102) and there is no need to deny the opportunity for young children to play with puppies if good hygiene is practised (29, 87).

Another association was found between *Toxocara* antibody titres and rural areas (36) or socio-economic factors like education attainment and income of head of the household (26, 34, 104).

Boys are sometimes observed to have a significant higher seroprevalence than girls (17, 36). Reasons for this might be related to differences in play and social behaviour.

Clinical symptoms, haematological findings and pathology

Larval migration through the body

After ingestion of infective *Toxocara* spp. eggs by the human, *Toxocara* larvae hatch in the stomach and migrate into the mucosa of the upper small intestine and penetrate via blood and lymphatic vessels throughout the body, resulting in somatic larvae in many types of tissues. The liver is an important site for controlling the migration of *Toxocara* larvae (liver entrapment). Dissemination occurs via the bloodborne route, through tissues and body cavities (86).

The migratory behaviour of larvae of *T. canis* and *T. cati* larvae is different. In mice, a relatively large proportion of *T. canis* was distributed to the brain (90), while in cats and mice the larvae of *T. cati* were mainly recovered from muscles, liver and lungs only rarely recovered from the brain (89). Experiments in rodents and non-human primates showed that

Toxocara second or third-stage larvae have a particular affinity for nervous tissue and may therefore be responsible for neurological disease in man (28, 29, 89, 90). Larvae do not migrate continuously, but tend to rest periodically before continuing their migration. During such periods of reduced movement, the larvae induce an immunologically mediated inflammatory response (86). Comparison of the distribution of somatic *T. canis* larvae in mice, 9 weeks and one year after infection, showed a decrease of somatic larvae in the carcass (41% to 30%) and an increase in the brain (56% to 68%) (3). Larvae in brains are not encapsulated and do not induce immunological reaction by the host. In most cases, no clinical disease will develop due to infection by small numbers of larvae.

Visceral larva migrans

A more marked, inflammatory, immune response is called visceral larva migrans syndrome or VLM (21, 26). This multisystem invasion can be associated with varied, non-specific clinical symptoms as a result of the host's immune response (20). VLM is mainly diagnosed in children between 1 to 7 years of age (mean age 2 years) and is characterised by persistent eosinophilia, leukocytosis, elevated γGT level and hypergamma-globulinaemia (12, 56, 80). Eosinophilia is seen more often in children than in adults (48). Clinical symptoms often include general malaise, fever, abdominal complaints (vague upper abdominal discomfort attributed to hepatomegaly), wheezing or coughing (17, 21). Larvae that remain in the liver can be associated with an eosinophilic granulomatous hepatitis. *Toxocara* infection should be considered in the differential diagnosis of any child with a persistent and unexplained eosinophilia (28) or recurrent abdominal pain (94). Toxocarosis can be considered as common, especially in children, and is associated with clinical features that are generally regarded as non-specific but together form a recognisable complex of symptoms (94). Chronic ‘idiopathic’ urticaria in adults and children is found strongly associated with the presence of antibodies to *Toxocara* and suggested to be caused by liberation of larval ES antigens (99). If subsequent exposure to *Toxocara* eggs is avoided, the disease is usually self-limiting (31). Severe clinical symptoms are reported including life-threatening pneumonia after massive infection (50) and eosinophilic meningo-encephalitis in children (62). Fatal cases as result of an exaggerated immunological response or extensive larval migration through the myocardium or central nervous system are rare (29).

Toxocara larvae have the ability to avoid the host’s immune responses and can survive in tissues for at least 10 years (23). The immune evasion is caused by the extraordinarily rapid turnover and shedding of the whole length of the outer surfaces of the larvae (‘dynamic larval

surface’). The inflammatory response is formed round shed surface components shed during larval migration (82). As result, the granulomata that form do not frequently contain histologically identifiable larval fragments (‘verminous tracks’). Another property of *Toxocara* larvae is the ability to exhibit host-like characteristics, for example A and B blood groups, on their outer surfaces. This occurs as the result of an active metabolic process rather than by the death and somatic degradation of larvae (83).

Ocular Larva Migrans

Migrating *Toxocara* larva(e) can induce granulomatous retinal lesions, which are characterised by complaints of loss of visual acuity, squint and 'seeing lights'. This is called Ocular Larva Migrans syndrome (OLM). In a minority of cases, total blindness of one or both eyes can result (23). Other authors indicate in contrast to this, that the presence of bilateral lesions suggests a diagnosis other than toxocarosis, because of the small chance that larvae will enter both eyes even in quite heavy infections (101). A commonly found clinical syndrome in OLM is a posterior pole granuloma mimicking a retinoblastoma. If not recognised, this can finally lead to enucleatio bulbi.

The mean age of patients with OLM is 8 years, but it is diagnosed in adults as well (80). The relative number of eye disorders may increase with age as well as the percentage of seropositive cases in a normal (subclinical) population (45) without particular association. Low numbers of larvae could escape the host immunity if provoked and finally reach the eye. Even low ELISA antibody titres may be indicative for OLM (52). The reported higher mean age of patients with OLM can be explained by a longer incubation period, because larvae can persist in the body for more than 10 years and periodically resume migration. Onset of clinical signs to a definitive diagnosis is described as 5.6 months and 22.6 months for VLM and OLM patients, respectively. Another explanation for the difference can be the difficulty of recognising visual impairment in young children (26). Ocular larva migrans is usually caused by no more than a single larva. It is apparent that patients with VLM indeed have higher *Toxocara* titres than those with OLM. The highest titres were found in the few cases in which OLM was associated with VLM. (26).

VLM or OLM syndrome

The distinction of human toxocarosis into visceral and ocular forms is an oversimplification, because patients with ocular manifestations will have been subjected to some degree of systemic migration by the infecting larvae earlier (23). Results of immunological testing of

intraocular fluids of OLM patients, obtained by using a micro-Ouchterlony test with different antigens, indicated an important role of *T. cati* in the epidemiology of larva migrans (71).

Toxocara infection rarely results in concurrent ocular and systemic disease. The differences between VLM and OLM suggest different pathogenic differences. Glickman and Schantz (26) explained these differences as the result of the number of infective larvae ingested. Lower doses of *Toxocara* are associated with a higher probability of OLM than VLM, because the antigenic mass of the larvae is insufficient to stimulate a protective immune response and allows them to migrate continuously. Ultimately this can cause OLM when entering the eye without evoking systemic signs. Antibody levels in dogs (26) and mice (43) were strictly dose-related and this may also be the case in man. Moderate to high infections will induce a strong immunological response, and most larvae will be destroyed or restrained in the liver. At very high doses larvae can overcome the filtering mechanisms of the liver and within a few days produce severe clinical signs, including ocular disease, which can be considered as concurrent VLM and OLM. Larvae in the eye could be recorded at necropsy in mice as early as 3 days after experimental infection (65).

Covert toxocarosis

A third clinical syndrome, called ‘covert toxocarosis’ (CT), was found in patients with clinical symptoms that are non-specific by itself and do not fall within the categories of VLM or OLM, but form together a vague complex of symptoms. Symptoms such as hepatomegaly, cough, sleep disturbances, abdominal pain, headaches and behavioural changes have been associated with raised *Toxocara* antibodies (93). In a group of French adults, such a disease was described clinically by weakness, pruritus, rash, difficult breathing, abdominal pain and allergic manifestations including eosinophilia and increased IgE levels (30). In older children beyond the toddler stage, the combination of abdominal pain, headache and cough was significantly associated with a high *Toxocara* ELISA titre than were individual clinical features (93). The diagnosis ‘Idiopathic Abdominal Pain of Childhood’ is usually made in children. The diagnosis of toxocarosis should be considered in children with cough and wheeze and having an additional history of headache and abdominal pain (93).

Concurrent diseases

Besides the complex of clinical symptoms discussed, there are other important arguments that support the achievement of an optimal prevention of *Toxocara* infection.

It was suggested that *Toxocara* larvae leaving the lumen of the intestine to migrate extensively in the tissues have the potential danger to be a vector of bacteria or viruses (100, 101), but published literature does not support this.

A causal relationship between *Toxocara* infection and the aetiology of epilepsy, as sometimes suggested (100), could not be established (10).

More recently a relationship between *Toxocara* seroprevalence and the incidence of asthma, elevation of serum IgE concentration, the presence of allergen-specific IgE and eosinophilia was established. Occurrence of asthma or recurrent bronchitis and hospitalization due to asthma were significantly related to seroprevalence, while eczema tended to be more frequent. It was concluded that allergic phenomenona in children who are predisposed to asthma, are more frequently manifested after *Toxocara* infection (8).

Toxocara larvae that enter the central nervous system may cause neurological disorders such as subtle neurological deficits or behavioural disorders in children (80). In one study (59), infected children performed worse on neuropsychological tests of motor and cognitive function than did uninfected controls.

An interesting finding was reported by van Knapen et al. (47), who detected antibodies to *Ascaris suum* in almost 50% of 430 *Toxocara* seropositive clinical patients in the Netherlands. Antibodies to both parasites were not observed in healthy individuals, although both infections occur frequently in the population. The hypothesis was presented that previous sensitisation to *Toxocara* or *Ascaris* followed by repeated infections may be necessary to induce overt toxocarosis or larva migrans syndrome.

A similar high percentage could not be established for double infection with *T. canis* and *Ascaris lumbricoides* in urban slum children in Venezuela. The highest percentage of double positives was 18.6% in the 7 to 9 year-old group (53).

Immunology

Infective *Toxocara* larvae induce a persistent immune response in humans, characterised by eosinophilia, leukocytosis and hypergammaglobulinaemia and the production of IgM, IgG and IgE antibody isotypes to TES and larval outer surface (80).

In mice, an infection with 5 eggs showed readily detectable alterations in eosinophil count and spleen weight-to-body ratios (43). Entrapment and killing of larvae in eosinophilic granuloma could be demonstrated in vivo and led to the conclusion that eosinophils and IgE may mediate larval killing. However, it was not possible, using fluorescence tests, to relate antibody binding to larval surfaces at physiological temperatures to either antibody titres to TES or to

other immunological criteria such as eosinophilia or lymphocytosis. Larvicidal effects could not be established in sera incubated with larvae at 37º (83). Animals with or without eosinophils were equally capable of liver trapping and specific IgE levels were not correlated with liver trapping capability, suggesting that the eosinophil and IgE components are not primarily responsible for this phenomenon (32).

It appeared that CD4+ cells are part of a complex effector system in liver trapping (32). Two distinct cytokine secretion patterns have been defined among CD4+ T cells. Type 1 (Th1) helper T lymphocyte produce Interleukin (IL)-2 and Interferon-γ (IFN), whereas Th2 cells express IL-4 and IL-5. Since IL-4 promotes the switching of B-cell isotypes to the production of IgE and IL-5 promotes the differentiation, vascular adhesion and survival of eosinophils, Th2 cells may be particularly implicated in the immune response to helminths. In *Toxocara* infected patients, high serum IgE levels and eosinophilia may be related to an increased number and activity of T cells with a Th2 pattern of cytokine secretion, with a concomitant reduction in the number of T cells belonging to the Th1-like subset. It is speculated that these are immunopathological consequences of the host-parasite relationship, rather than a successful effector mechanism against the worm (14).

The surface exposed epitopes of viable larvae and TES are sugar-rich and it has been hypothesised that antibodies with anti-carbohydrate paratopes are the most likely to mediate surface binding. The reaction of the immune system depends on this. The anti-polypeptide paratopes of antibodies will bind less to the carbohydrate-rich larval surface, but predominantly to the polypeptide epitopes in TES depots. This will result in reduced larval entrapment and antibody dependent cytotoxicity allowing more larvae to survive and as a consequence of the increased tissue deposition of TES due to an inability to kill larvae, an increase in pathological effect. Twice as many of the sera from Covert toxocarosis patients had low IgE titres to TES (85) and IgE paratopes from CT-patients recognising polypeptide epitopes of *Toxocara* larvae, as compared to IgE from sera of VLM/OLM patients. IgE from CT sera therefore binds less readily to larval outer surfaces with the likely outcome of increased pathology. This could explain the increased inflammatory response and broad range of clinical signs and symptoms in these patients (86).

Diagnosis

Direct diagnosis of *Toxocara* infection is not easy, because patients do not excrete parasite material such as eggs or larvae, while migrating larvae are not easily found in biopsy material. An accurate histopathological diagnosis of larval toxocarosis in biopsy material is possible with a staining technique described by Parsons et al. (68) that shows patterns of antigen deposition in acute infections and in granulomas, empty or centred around larvae.

Serodiagnostic techniques are reliable tools to detect antibodies and circulating antigens. The value of immunodiagnostic tools in sero-epidemiology largely depends on the positive predictive value of the test system used. This value in turn is dependent on the prevalence of an infection in the population and the specificity of the method used (48). *Toxocara* infective stage larvae produce *Toxocara* excretory-secretory (TES) antigens (76) or larval extracts (31). These products, of in vitro maintained second stage larvae, are used for a *Toxocara* ELISA with a reported high specificity of 92% to 95% and sensitivity of 73% to 78% at a diagnostic titre of 1:32 (25, 72). Indirect ELISA's were used to determine the prevalence of total IgG, IgE and IgM antibodies to TES (86).

The ELISA titre is also reported as optical density units by the Toxocara Reference Laboratory in London. (95).

Not all studies, however, reported the levels they considered to be positive, which makes comparison difficult. Various starting dilutions of serum are used in different laboratories (35) and methodology may vary from laboratory to laboratory. The populations surveyed are not always uniform. Most are populations of children attending hospitals instead of surveys of groups such as blood donors that are more representative of the 'normal' population. Other diseases may affect *Toxocara* titres (95). Cross-reactions due to other parasitic infections should be excluded, including other migrating larval infections (45). The toxocaral ELISA test has to be interpreted with some caution because TES products contain parasite-derived human A and B blood group like substances, which might complicate the interpretation of the test when human sera with anti-A or anti-B isohaemagglutinins are tested. Further purification was necessary to restore the validity of the tests (84). To avoid this interference, an anti-*Toxocara* IgE ELISA test was suggested as the most useful serodiagnostic test, because it will not detect iso-haemagglutinins in the test sera as these are largely of the IgG class (23). Testing for isotype-specific antibodies (sIgE) to TES without interference of IgG antibodies is used (55, 86), but the specificity and sensitivity was moderate and the sIgE ELISA was considered to be insufficient to allow confirmation of the serodiagnosis of toxocarosis when used alone. It might be interesting as a complementary method for the detection of specific IgG (55). Not all patients with elevated total IgE levels demonstrate specific anti-*Toxocara*

IgE, although the presence of specific IgE is likely to be associated with an active infection (48).

Other methods for developing specific assays for the diagnosis of larva migrans are IgM monoclonal antibodies which recognise species- and genus-specific epitopes (7) or a specific Ouchterlony's diffusion-in-gel method using adult worm extracts (64). Promising seems a recently reported very sensitive polymerase chain reaction to detect DNA of *T. canis* in liver tissues of experimentally infected mice (105).

The Western-blotting procedure (testing specific IgG) for the immunodiagnosis of human toxocarosis is used with high sensitivity and specificity, avoiding problems of cross-reactivity with sera infected with other helminth diseases (54).

In experimentally infected animals antibody responses to TES antigens becomes detectable 4 days to 4 weeks after infection and can persist for months to years (11, 44, 28). As few as 5 infective eggs can produce symptoms and seroconversion in mice (23, 43).

Finding a positive serum titre is not always proof of a causative relationship between *Toxocara* infection and the patient's current illness. In many cases it reflects the prevalence of asymptomatic toxocarosis. It should also be emphasised that serological prevalence is not synonymous with infection rate, because it depends on the sensitivity and specificity of the serological method used to quantify the antibody response (74).

The serological tests for OLM have a lower sensitivity, probably as result of low larval burden and/or the longer period between infection and testing. The mean period between onset of illness and serodiagnostic testing was less than 6 months for VLM and 2 years for OLM (77). In a small proportion of OLM patients, antibodies cannot be detected in serum and in rare cases, if larvae migrate through the ocular tissues, they can be visualised using ophthalmology. Negative serological results and normal blood eosinophilia are due to a physiological barrier between blood and ocular fluids (immunological blood-eye barrier) (71).

A solution to provide a definitive diagnosis would be the demonstration of antibodies in the vitreous humour (5) using the ELISA TES-test or the micro Ouchterlony test that requires only small amounts of ocular fluid (10 to 20 µl) (71).

Criteria for the diagnosis of OLM are formulated by Petithory et al. (70) as positive immunological tests for nematode antigens and eosinophilia of vitreous or aqueous humours and the presence of ocular lesions.

Epidemiology

The exposure to *Toxocara* spp. in the Netherlands, based on serologic surveys, was found to be 19% on average: between 4% and 15% in people younger than 30 years and 30% in adults older than 45 years. (61). Regularly re-infection of adults is probably the cause of the higher prevalence (45). Titres fall gradually over a period of about three years, but should be considered as a balance between the fading memory of the immune system and its stimulation by continuing ingestion of viable ova or reactivation of dormant larvae (95).

Seroprevalence for *Toxocara* in some other countries varied between 4.6 to 7.3% in children in the USA (34), 2.5% in Germany (51) to 83% for children in the Caribbean (96). In tropical climates transmission is probably favoured by high ambient temperature and humidity. An expanded review of cases of toxocarosis (VLM and OLM) from all over the world (17) revealed that more than half of the patients were less than three years old, one fifth were adults and sixty per cent were males. In a survey in Scotland (22), 16% of patients with unexplained eosinophilia and hepatomegaly, 15% of patients with ocular lesions and 14% in cases of hayfever, asthma or eczema tested positive for *Toxocara*. In a recent study in Spain (18) sera of a selected group of patients with clinical symptoms such as eosinophilia, splenomegaly and recurrent pain and asthma were assayed for *Toxocara* antibodies using the ELISA method. The results were that 23% of 30 adults, 33% of 332 children and 18% from 45 patients of unknown age tested positive.

Many people infected with *Toxocara* are undiagnosed because signs are absent or non-specific and serological tests are not requested for most patients (80). In a recent study in the Netherlands, only 8% of general practitioners surveyed were said to have requested *Toxocara* serology for their patients in the past (66).

OLM cases in the Netherlands over the last decade did not exceed 10 cases (annual incidence 1:15 million) as estimated by the Netherlands Ophthalmic Research Institute, National Uveitis Workshop (Personal communication Prof. Dr. A. Kijlstra, Chairman, 1993).

Methods of control

Preventive measures

A zoonotic disease like toxocarosis can be prevented for the most part. Control is important from the point of view of welfare, for the quality of human life and also for the economic costs to society. These costs can be estimated depending on the incidence of clinical disease, estimated number of physician visits per patient, hospitalisation rate and average length of

stay in clinically significant disease, the costs of pharmaceuticals and income losses (91). The mean cost of an adult toxocarosis patient (medical expenses and loss of days of labour) was estimated in 1989 as £ 620 (56).

Prevention of toxocarosis is possible by the institution of certain measures: appropriate health care for pets including regularly anthelmintic treatments, reducing the number of uncontrolled and stray pets, preventing contamination of the environment with faeces and promoting responsible pet ownership (38, 91).

To increase awareness of particularly pet owners about the potential zoonotic hazards, veterinary practitioners, general practitioners and public health agencies should provide sufficient information and advice in order that appropriate measures can be taken to minimise the risk of infection. Several reports, however, have indicated a significant lack of knowledge within the professions (33, 49, 67). All authors concluded that continuing education with emphasis on the zoonotic risks is still strongly recommended.

Treatment of patients

Patients with severe *Toxocara* infections, particularly if there is central nervous system involvement, can be treated with systemic acting and larvicidal anthelmintics (23, 57). Effective results are reported with use of diethylcarbamazine (13, 58), albendazole, oxfendazole, cambendazole (1), fenbendazole (16, 98), mebendazole (58) and levamisole (97). Most of these results are obtained from experiments on animals (mice) where administration of higher doses of anthelmintics started directly after inoculation with *Toxocara* larvae (mimicking acute infection) and continued for several days.

Clinicians should balance the risk of therapy with the severity of the disease, because treatment can lead to severe hypersensitivity reactions caused by dying larvae. Toxic reactions of the used anthelmintics can occur especially in OLM cases (9, 58). The anthelmintic dose should be increased gradually over a period of days and covered by the concomitant administration of steroids (23). Good results and a low rate of adverse reactions in man is described for mebendazole at a daily dose of 20-25 mg/kg for 21 days (58).

Laser photocoagulation can be used if OLM lesions are located (19). Marked allergic reactions or an inflammatory reaction, for example in the eyes, can be suppressed with systemic or local corticosteroid therapy (57) without the risk of enhancing the infection, because multiplication of larvae is not possible.

The immunological mechanisms of human Th1 and Th2 cell subsets may provide a novel means of therapy of helminth infections, including selective inhibition of Th2 cell activity

and/or amplification of Th1-like responses. Th1 cells are important effector cells in inflammatory reactions associated with delayed-type hypersensitivity responses and low antibody titres, while Th2 cells may account for both the persistent production of antibodies, including IgE (via IL-4), and eosinophilia (via IL-5) observed in helminth infections or other IgE-mediated disorders, such as atopic diseases (14).

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Chapter 3

Prevalence of intestinal nematodes of dogs and cats in the Netherlands

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Abstract

Faecal samples from 272 dogs and 236 cats from Dutch households were examined for nematode eggs. *Toxocara* eggs were found in 8 dogs (2.9%) and 11 cats (4.7%). *Toxascaris* eggs were found in 1 dog (0.4%) and *Trichuris* eggs in 2 dogs (0.7%). Examination of faeces from 56 stray cats revealed *Toxocara* in 12 cases (21%) and *Toxascaris* eggs in 3 cases (5.4%). No hookworm eggs were found. The percentage of positive samples was significantly higher in young animals than in older animals. *Toxocara* eggs were found significantly more frequently in stray cats than in cats kept in households.

Introduction

The intestinal helminths described in dogs and cats in the Netherlands include *Dipylidium caninum* (dog, cat), *Toxocara canis* (dog), *Toxocara cati* (cat), *Toxascaris leonina* (dog, cat) and *Trichuris vulpis* (dog) (3, 12). *Toxocara* spp. infections in dogs and cats have, because of their zoonotic significance, important public health consequences. In the Netherlands, between 7% (children) and 20% (adults) have antibodies against *Toxocara* (2). A survey in Utrecht (7), revealed contamination levels of *Toxocara* of 9% (0-20%) in public parks (mainly *T. canis*) and 49% (33-63%) of sand-boxes (mainly *T. cati*). Nearly all eggs were embryonated. These findings are consistent with reports from other European cities. Since the actual prevalence of *Toxocara* infestation in Dutch dogs and cats is unknown, surveys of dogs and cats in Dutch households in two different urban areas and one survey of stray cats were performed to determine the prevalence of intestinal nematodes.

Materials and methods

Sampling areas

Group 1. Amersfoort

During the period January to March 1993 a total of 207 households with 133 dogs and 148 cats were visited in Amersfoort and surrounding villages to investigate the prevalence rates of intestinal nematodes and eventual regional variations. Every region was equally divided into several parts after which households in some streets per region were chosen at random for a visit. Households were asked about the use of anthelmintics. Faecal samples were collected

from 121 dogs and 76 cats for examination. The average age of these animals was 5.0 and 7.2 years respectively. The sex distribution of the total group of animals was equally divided and 42 dogs (35%) and 67 cats (88%) were neutered or castrated. All dogs and 60 cats (79%) went outdoors. Three dogs (2.3%) and five cats (6.8%) had been used for breeding at least once during the last five years.

Group 2. Utrecht

A similar survey was performed in the city of Utrecht and some surrounding villages during the period August 1993 to February 1994. In total 253 households were visited (comparable procedure to Group 1). Faecal samples were collected from 151 dogs and 128 cats, for faecal examination. The average age of these animals was 6.1 and 6.5 years respectively. Females were in the majority for both dogs (54%) and cats (58%). Nearly all dogs (148, 98%) were able to go outdoors, whereas 73% (n=93) of the cats could do so. The composition of this group of animals was comparable with that of Group 1.

Group 3. Cats from cat boarding houses

From the surveys it became clear that it was almost impossible to collect faeces from cats that defaecate outdoors. For this reason, four cat boarding houses in the region Amersfoort were visited during the summer of 1993 to obtain faeces from cats which normally defaecated outdoors. In total 32 faecal samples were collected. The sex distribution of the cats was equally divided (16 of each) and the average age was 6.1 years.

Group 4. Stray cats

During the summer of 1993, veterinary practitioners from shelters in the cities of Amsterdam, Utrecht, the Hague, Rotterdam, and Nijmegen collected faeces from 56 stray cats. These animals were caught in cages all over the city for neutering. For 52 cats the sex was noted (20 female and 32 male). The mean age of 50 cats was estimated (based on size and teeth) as 2.6 years. Information about the condition of 53 cats was available: 41 cats were in good or very good condition (80%), the remaining cats being in reasonable (n=11) or bad (n=1) condition.

Faecal examination

Faeces were stored in plastic tubes frozen at -20° C until examination (within 2 weeks). Samples from stray cats were collected in SAF fixative solution (sodium acetate 1.5%, acetic acid 2% and formaldehyde 1% in purified water). Of these samples, 1 g of faeces was

suspended in 135 ml tapwater and sieved through a sieve with a mesh width of 250 µm. The sample was centrifuged for 1 minute at 3000 rpm (RCF 37.5). The supernatant was decanted and the sediment was suspended in a $ZnSO_4$ solution with a specific gravity of 1.3 g/ml. A cover slide was placed on the tube and centrifuged for 1 minute at 3000 rpm. This slide was examined by light microscopy at a magnification of 40 - 100. The eggs were identified and counted and results expressed as Eggs Per Gram (EPG).

Statistical analysis

The presence of eggs in different regions and in different groups of animals was statistically analysed by using 'Yates corrected Chi Square' in Statistix 3.5 (Analytical Software), applying a significance level of 5%.

Results

No difference in results could be detected between animals kept either in the central town area, in the adjacent villages, or in rural areas in group 1 or in group 2. Therefore the results were summed for the different groups.

The egg counts of **group 1** are listed in Table 1. Of 121 dog faecal samples, *T. canis* eggs were found in 4 (3.3%) and *T. vulpis* eggs in 1 (0.8%). Two (2.6%) of the 76 cats were shedding *T. cati* eggs. No relationship could be established between positive animals and anthelmintic treatment, opportunity of going outdoors, age, or sex.

Four (2.7%) of the 151 investigated dog faecal samples from g**roup 2** had *T. canis* eggs, one of which (0.7%) also had *T. vulpis*. In one (0.7%) sample *T. leonina* eggs were found. In seven of the 128 cat samples (5.5%) *T. cati* eggs were found (Table 1). Dogs and cats younger than 1 year of age in Group 2 were significantly more often infected than older animals (p value respectively <0.01 and $p<0.001$). No relationship could be established between worm infection, opportunity of going outdoors, sex, and anthelmintic treatment (routine worming was performed in 44% of the animals with an average frequency of 1.5 treatments per year).

In **group 3**, three of the 32 cat faecal samples (9.4%) were positive for *Toxocara* eggs (Table 1). No significant difference was found between worm infection and anthelmintic treatment, sex or age. The prevalence was not significantly higher than the prevalence in cats in groups 1 and 2. Only 4 of the 30 animals in this group were wormed regularly.

From the stray cats in **group 4**, 12 out of 56 faeces samples (21%) had *Toxocara* eggs and 3 (5.4%) were positive for *Toxascaris* eggs (Table 1). No relationship was found between worm infection and sex, condition or estimated age. The prevalence was significantly higher than the prevalence in cats in group 1, 2 and 3 ($p<0.0001$).

group	species	numbers tested	numbers positive	age (years) average	age (years) range	male sex	numbers with A	B	C	D[a]
1	dogs	121	5 (4.1%)	0.8	0.2 - 1.5	3[b]	4	-	1	-
	cats	76	2 (2.6%)	7.0	5.0 - 9.0	2	-	2	-	-
2	dogs	151	5 (3.3%)	2.4	0.5 - 6.0	4	4	-	1	1[c]
	cats	128	6 (4.7%)	2.0	0.1 - 7.0	3	-	6	-	-
3	cats	32	3 (9.4%)	2.0	1.0 - 3.0	0	-	3	-	-
4	cats	56	15 (26.8%)	1.9[d]	0.5 - 4.0	6[e]	-	12	-	3

[a] A: *T. canis* B: *T. cati* C: *T. vulpis* D: *T. leonina*

[b] The sex of one animal was not recorded

[c] One combined infection of *T. canis* and *T. vulpis*

[d] Estimated age

[e] The sex of three animals was not recorded

Table 1. Specification of dogs and cats with worm infections in this study

Discussion

Before comparing the results with those of other reports, one should consider the sensitivity of the floatation test. Results published by Lillis in 1967 (9), and confirmed by Vanparijs in 1973 (14) and Fok in 1988 (4), showed a significantly higher prevalence of helminths after post-mortem examination than after faecal examination.

This difference may be explained by light infections, the presence of male worms only, the presence of immature non-egg producing stages of worms (often *T. canis*), and intermittent egg shedding. These causes are related to the investigated animals and are independent of the test used. Lillis examined 2737 samples from dogs and 1480 from cats by the faecal floatation method ($ZnSO_4$ sp. gr. 1.2) and autopsied 685 dogs and 318 cats from these groups and calculated an average overall sensitivity of 80.1% for the different worm species (50.0 - 99.1%). There was a sensitivity of 0.51 for *T. canis* and a specificity of 1, a positive predictable value of 1, and a negative predictable value of 0.88. Therefore the actual

prevalence in our study can be roughly estimated as being higher (maximal double) than the apparent prevalence. Although the faecal floatation test can be considered reliable for all helminth eggs, the goal of our study was primarily to look for nematode eggs.

The results of our study differ markedly from those of other studies (Table 2). In the Netherlands, Rep (11) published in 1980 the results of a survey involving 544 adult dogs during the period 1972 - 1977. Apart from the fact that these results are relatively dated, the results were not representative of the total Dutch dog population since only adult experimental dogs were investigated. Moreover, larger breeds were over-represented and the animals were obtained from commercial dog dealers all over the country. This is considered as a selected sample and a relatively higher infection rate can be expected when dogs from several origins are brought together by dealers. In addition, Rep used a different floatation method (with NaCl), no analysis of anthelmintic strategy by the dealers was performed, and no cats were investigated.

reference	**species**	**numbers tested**	**overall prevalence**	**prevalence of intestinal helminths[a]**					
				A	**B**	**C**	**D**	**E**	**F**
Rep[11]	dogs	544	22.9	14.5	-	-	11.2	-	-
Vanparijs[13]	dogs	2324	34.2	17.4	-	7.0	10.1	11.4	2.1
	dogs	246	36.1	12.2	-	24.7	4.5	8.5	-
	cats	30	83.3	-	60.0	-	-	36.6	20.0
Hörchner[6]	dogs	422	28.4	10.4	-	2.4	6.6	1.7	3.1
	dogs	605	7.1	3.5	-	1.2	1.3	0.2	0.2
Hinz[5]	dogs	155	20.0	5.8	-	3.2	3.9	3.2	5.2

[a] A: *T. canis* B: *T. cati* C: *T. vulpis* D: *T. leonina* E: hookworms F: cestodes

Table 2. Comparative studies of worm infections in dogs and cats

Another explanation for the higher prevalences in the study of Rep than in our study could be the increased availability of better anthelmintics after 1980, together with a higher worming frequency by owners as result of awareness of possible worm problems and/or more responsible pet ownership.

More recently, a survey of more than 2000 stray dogs, 246 kennel dogs, and 30 cats during the period 1980-1990 in Belgium was published (13). The results were extrapolated to the Belgian dog and cat population and suggested high prevalences of worm infections in dogs and cats. Assuming that there will be no influence due to geography, the impression could be created that the overall prevalence of worm infections is increasing and that more worm species are involved. However, in the study of Vanparijs et al. (13), the animals were also obtained from dealers, sex and age were not reported, nothing was mentioned about anthelmintic treatments by the dealers, and the 246 kennel dogs were obtained from four kennels (an average of 60 dogs per kennel), which can give rise to an increased risk of worm infection. Finally, a relatively small number of cats were involved and two different floatation methods (NaCl sp.gr. 1.2 and the McMaster egg count method) were used.

Nematode prevalences of dogs in Germany were published by Hörchner (6) in 1981 and Hinz (5) in 1985. Hörchner investigated faeces from 422 dogs in veterinary clinics in Berlin from 1978 and 1980 and 605 dog faecal samples obtained from the streets. A floatation method with a combination of $ZnCl_2$ and NaCl was used. The first group was not considered as representative, and contained a lot of young dogs visiting the clinic for their first vaccination. The second group, however, can give a more random impression of the worm infection in dogs in Berlin and showed considerably lower prevalences of nematode infections. Hinz took a representative sample of 155 dogs from the officially registered dog population of 207 animals in a more rural village area in the Hessian Neckar Valley and used an acid-ether concentration method. Nothing was mentioned about the time of the year when the samples were collected. Finally, a more recent impression of the prevalence of *T. canis* in dogs in the Netherlands was obtained by Jansen et al. in 1993 (7). Of the dog faeces (n=108) collected in parks, 7% were positive for *T. canis*. This percentage is not more than an indication, because nothing was known about the composition of the group of dogs. Although hookworms have not been detected in dog faeces in The Netherlands so far, *Uncinaria stenocephala* was found in 60% of the faeces of wild foxes (1). It should be borne in mind that nowadays foxes sometimes live in urban areas.

From the field survey in randomly chosen households in Utrecht and Amersfoort in the present study, it can be concluded that the prevalence of intestinal nematodes in Dutch dogs and cats is not as high as would be expected from earlier Dutch and Belgian reports, but is more comparable to the prevalence reported in German studies. The prevalence of worm

infections in dogs and cats in Dutch households in our study did not exceed 5% and no differences could be detected between city quarters and more rural areas with a different socio-economic status of owners. But the present study also has its limitations. To be able to draw conclusions for the total dog and cat population, based on these low prevalences, would require a sample size of nearly 7000 dogs and cats (10) (total population 1.2 million dogs and 2.2 million cats in the Netherlands, confidence interval 1.96 (95%), estimated prevalence 5%, and an accepted variation of 0.5%). It is practically impossible to examine this number of animals. The results of this descriptive epidemiological study should therefore be considered as indication for a low prevalence of nematode infections in the Dutch domestic dog and cat population. Some bias might have been introduced by the time of the year during which the samples were collected (August until April) and the non-response percentages of the participants. Owners who have a less responsible attitude to pet care were probably not willing to participate in this study. The non-response rate however was very low (< 5%). Our results are similar to those reported by Jordan et al. (8) for the U.S.A. In 21,583 faecal samples collected from dogs that visited the Oklahoma State University Clinic during 1981 - 1990, a prevalence of 4% for *T. canis* was found in 1990, the prevalence of *T. vulpis* infections was much higher, 9%, in 1990.

It is important for human health reasons to know which animals are responsible for the contamination of the environment with *Toxocara* eggs. Stray dogs are very rare in the Netherlands and, if found, are placed in animal shelters. The infectious dogs and cats (especially the younger ones) detected in the households surveyed will certainly shed eggs in parks and sand boxes. All dogs and nearly 80% of the cats were able to go outdoors. Stray cats showed a significantly higher rate of infection with *T. cati* (21%) than pet cats and can therefore be considered as an important source of contamination of the environment with nematode eggs.

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Chapter 4

Nematode infections in dog breeding kennels in the Netherlands, with special reference to *Toxocara*.

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Abstract

Faecal samples from 286 adult dogs and 159 pups and dust and soil samples from 32 dog breeding kennels in the Netherlands were examined for nematode eggs. Dogs that shed nematode eggs were found in 41% of the kennels. The kennel prevalence of nematode infection of adult dogs was 33%. The kennel prevalence for infection of adult dogs and pups with nematode species was 21% and 48% for *Toxocara canis*, respectively, 29% and 0% for *Trichuris vulpis*, and 20% and 0% for *Toxascaris leonina*. Kennels with more than two litters per year and with regular import of new animals had a significantly higher prevalence of *T. canis* ($p<0.01$ and $p<0.05$ respectively). *T. vulpis* infections in adult dogs occurred significantly more often in kennels that used deworming products other than benzimidazoles ($p<0.05$). Embryonated *T. canis* ova were recovered from 20% of the house and kennel dust samples and from 50% of the soil samples. This survey shows that the nematode infection rate in dog breeding kennels is high. Better deworming strategies should be used to improve the health status of the dogs and to reduce the risks of zoonotic infection in humans.

Introduction

The intestinal helminths described in dogs in the Netherlands include *Dipylidium caninum*, *Toxocara canis, Toxascaris leonina,* and *Trichuris vulpis* (6). Toxocarosis is an important zoonosis (16, 21) caused by *Toxocara canis* and *Toxocara cati,* parasites of dogs and cats, respectively. Some variables that play a role in the epidemiology of *Toxocara* infections in humans in the Netherlands (5) are soil contamination (14), and infection of companion dogs and cats and stray cats (17); but breeding kennels were not included in these surveys. The present study was performed to determine the prevalence and risk factors of gastrointestinal nematodes, in particular *T. canis,* in dog breeding kennels.

Material and methods

Sampling

At a national congress of dog breeders, 32 Dutch dog breeders were recruited to participate in a survey on worm infections in dog breeding kennels. The kennels, situated throughout the country, were visited between January 1994 and August 1995. Faecal samples (> 10 g) from all 286 adult dogs and 159 pups present were collected. The dogs were of 29 different breeds. Dogs younger than 6 months were considered as pups. Dogs were able to exercise outdoors in dirt runs in 22 of the 32 kennels. Soil samples (± 500 g) were taken with a garden trowel to a depth of 5 cm from three random locations in these runs. Dust samples were taken (with a hand-held vacuum cleaner) from rooms in the breeders' homes, from floors, carpets, baskets (± 4 g), and (or) from indoor kennels (± 35 g). These samples were collected from 16 of the 32 kennels. Faecal, soil, and dust samples were stored at -20º C until examined. Data about the animals' housing, nutrition, cleaning, disinfection, and use of anthelmintics were compiled.

Techniques

Faecal examination was performed with a sedimentation-flotation technique, using a $ZnSO_4$ solution (specific gravity of 1.3 g/cm^3). The sensitivity of this test was 10 eggs per gram (17). Although faecal flotation test can be considered reliable for all helminth eggs, the goal of our study was primarily to look for nematode eggs.

Soil samples were thoroughly mixed and sub-samples of 300 g were used for further analysis. To each of these samples 1 ml of anionic detergent (Teepol®) was added and the samples were diluted to 1000 ml with tap water. The samples were stirred manually several times for 2 hours and finally sieved with running tap water over successive sieves with pores of 1000, 300, 150, 106 and 38 µm in a sieve shaker (Haver and Boecker EML 200). The retentate on the 38 µm sieve was washed into a beaker and allowed to stand for 1 hour. The supernatant was drawn off and the total sediment was processed by using the sedimentation-flotation technique referred to above.

Dust samples were weighed and 1 ml of anionic detergent (Teepol®) was added. Samples were diluted up to about 100 ml with tap water and stirred manually several times for two hours. This preparation was sieved through a 1000-µm sieve to remove hair and other detritus. The filtrate was processed by using the sedimentation-flotation technique referred to above.

Eggs of *Toxocara* in soil and dust samples were differentiated by the fine pitting of the superficial layer of *T. cati* eggs compared to that of *T. canis* eggs.

General description of the kennels

The average number of adult dogs per kennel was 9 (range 1-30). Pups were present in 63% (20/32) of the kennels, with an average number of 7.7 pups (range 1-34). The annual number of litters was 2.5 (range 0.5-10). Seventeen breeders (53%) claimed to maintain a closed population; the others regularly introduced new dogs. With one exception, all dogs were group-housed. The floor of the indoor living areas of the dogs was of concrete, flagstones (25 kennels), wood or cork (3x), or linoleum (3x). In one kennel the dogs lived entirely outdoors (Newfoundlanders) on grass. All the remaining breeders exercised their dogs daily on concrete floor runs (9x), grass runs (19x), or on sand (3x). Of the 31 breeders with indoor kennels, 17 washed these daily, 12 weekly, 1 monthly, and 1 never (only removing the faeces daily). A detergent was used in 13 kennels; the remaining 17 kennels regularly used disinfectant, including 7 that used a commercial chlorine bleach. At parturition, 5 breeders (16%) confined whelping bitches in a kennel, 7 (22%) in the owners bedroom, and 20 (62%) in the living room or kitchen. Thirty breeders fed their dogs on commercial dry food, and 7 of these alternated dry food with cooked meat. Two breeders fed their animals on raw meat only. Adult dogs were dewormed on average 2.3 times (range 1-6) per year. Twenty-three breeders (72%) gave an anthelmintic treatment to bitches during oestrus or pregnancy and 20 (63%) also post partum. Pups were dewormed 2.7 times (range 1-4) during the first 12 weeks of life. Ten breeders (31%) complied with the recommended Netherlands deworming schedule by treating pups at 2, 4, 6, and 8 weeks of age (3). The average age of first pup deworming was 3 weeks but in 34% (11/32) of pups deworming occurred at 3 weeks or later.

The following active ingredients were used for deworming adult dogs and pups respectively: flubendazole (0x/8x), levamisole (2x/2x), mebendazole (5x/0x), nitroscanate (8x/0x), oxibendazole (9x/11x), oxfendazole (0x/1x), piperazin (3x/0x), pyrantel (0x/10x), pyrantel/febantel combination (4x/0x), and unknown (1x/0x).

Statistical analysis

Statistical analysis was performed by univariate contigency table analysis, using Yates correction in Statistix 3.5 (Analytical Software), and applying a significance level of 5%. Analyses were done using the kennel as the factor to determine the significance of risk at the

kennel level. Risk factors at the animal level were determined by using the dog as the experimental unit.

Results

Faecal samples

The results of the faecal examination are summarized in Table 1. Nematode eggs were detected in 80 of the 445 faecal samples collected in 13 of the 32 kennels. *Toxocara* eggs were found in 22 samples from 286 adult dogs collected in 10 of the 32 kennels, and in 24 samples from 159 pups collected in 7 of the 20 kennels (in which pups were present). Adult dogs and pups with *T. canis* infection were aged 3.5 years (range 0.75-8) and 7 weeks (range 4-18), respectively. Fifty-five per cent of the infected dogs were female. *T. vulpis* eggs were found in 32 samples from 90 adult dogs collected in 7 kennels but were not found in any pups. *T. leonina* eggs were found in 3 adult dogs in 1 kennel, but were not found in pups.

parasite	**animals**	**samples positive (%)**	**kennels positive (%)**	**% infected dogs in infected kennels**
all nematodes	adults dogs & pups	80 (18%)	13 (41%)	33% (4-92%)
Toxocara canis	adult dogs pups	22 (8%) 24 (15%)	10 (31%) 7 (35%)	21% (3 - 60%) 48% (11 - 100%)
Trichuris vulpis	adult dogs pups	32 (11%) 0 (0%)	7 (22%) 0 (0%)	29% (13 - 67%) 0%
Toxascaris leonina	adult dogs pups	3 (1%) 0 (0%)	1 (3%) 0 (0%)	20% 0%

Table 1. Presence of parasite eggs in the faeces of 286 adult dogs and 159 pups in 32 kennels

Soil and dust samples

The results of the examination of the dust and soil samples are provided in Table 2. *Toxocara* eggs were present in dust from 21% of the breeders' homes and in 20% of the kennels. Half of the 22 soil samples tested positive for *T. canis* eggs and 3 (14%) samples had *T. vulpis* eggs. With one exception, all positive soil samples contained embryonated eggs. *Heterakis* and

Capillaria eggs were found in one soil sample and another contained eggs of an Anoplocephalid cestode. These findings were attributed to the concomitant presence of poultry and goats, respectively.

source	**samples**	***Toxocara* positive**	**%**	***Trichuris* positive**	**%**
soil	22	11	50%	3	14%
kennel dust	10	2	20%	--	0%
household dust	14	3	21%	--	0%

Table 2. Presence of parasite eggs in samples of household dust, kennel dust, and soil.

Risk factors

At a kennel level, nematode infections were significantly more frequent (p=0.035, 1df) in open kennels (regular import) than in closed kennels. *T. canis* infections were found more frequently (p=0.008, 1 df) in kennels in which there were two or more litters annually than in kennels with fewer litters. *T. vulpis* was found significantly more frequently (p=0.026, 1 df) in kennels which used non-benzimidazoles (6/13) than in kennels that used benzimidazoles for deworming (1/18). Information was not available on the use of anthelmintics for one kennel.

At the animal level, no significant relation was found between the frequency of worm infections and factors such as the number of dogs per kennel, frequency of deworming, cleansing with chlorine products, where the bitches whelped, and the structure of the floors of the kennels. There was no significant difference in the incidence of *T. canis* infection between pups whelped by bitches dewormed during pregnancy and pups from bitches that were not dewormed during pregnancy.

Discussion

The results of this study show that kennel dogs are frequently infected with nematodes. The experimental population can be considered a convenience sample of the reference population, because the dog breeders volunteered, which may have biased results. However, this is unlikely because the study included large numbers and types of breeders and breeds from all over the country. *Toxocara* infections were seen in 8 to 15% of the kennel dogs (adult and

pups respectively). These values are higher than the 7% *T. canis* positive faecal samples collected from public parks (14) and the 2.2% prevalence of dogs from households (17). The frequency of *Trichuris* infections was rather high in the kennel dogs because this parasite was not found in the faecal samples from public parks and occurred at a much lower frequency (0.7%) in dogs from Dutch households. *Toxascaris* infections were seen in 1% of the kennel dogs in this survey but were not seen in samples from public parks and in only 0.4% of dogs from Dutch households. These findings confirm the hypothesis that infection pressure from canine intestinal parasites is higher in kennels than in individual domestic situations. The higher frequency of *Trichuris* infections can moreover be a consequence of the use of anthelmintics that are not effective, or that are less effective against this whipworm, including levamisole, nitroscanate, piperazine, and pyrantel (1). In contrast, incorrect use of effective anthelmintics in inappropriate deworming schemes will also influence the prevalence of *Trichuris*.

Surveys of kennels for military dogs in France (7), racing Greyhounds in the UK (12), show dogs (11), and police dogs in training schools (19) showed various prevalences of these nematodes (Table 3).

reference	**numbers tested**	**overall prevalence**	**prevalence of intestinal nematodes**			
			T. canis	***T. vulpis***	***T. leonina***	***U. Stenocephala***
Jacobs & Pegg,1976	574	12.2%	7.3%	1.9%	2.8%	0.9%
Jacobs & Prole,1976	572	49.3%	15.4%	6.3%	15.4%	33%
Pegg 1978	808	13.2%	5.5%	0.5%	5.5%	0.6%

Table 3. Prevalence of nematodes in kennel dogs

Hookworms were found in all these studies, but were not found in our study. The prevalence of *T. canis* and *T. vulpis* in some heavily infected Greyhound breeding kennels in the Midwestern United States was 3.4% and 1.3%, respectively, in dogs older than 12 months and 24% and 0%, respectively, for dogs younger than 12 months (20). Ova of *T. canis* and *T. vulpis* can survive in contaminated soil for years (9). The risk of nematode infection in kennels can be increased by the regular whelping of pups infected with *Toxocara* and the existence of many such animals can contaminate intensively used exercise runs.

T. canis eggs were recovered from 20% of the dust samples in the house and kennels and 50% of the soil samples in our study, and 14% were positive for *T. vulpis*. Jansen en van Knapen

(14) found a much lower frequency of *T. canis* eggs in Dutch public parks. This finding supports the hypothesis that infection pressure is higher in kennels than in other environments. Similarly, half of the soil samples from outdoor training areas of English police dogs contained *Toxocara* and 4% had *T. vulpis* ova (19). Ninety five per cent of the eggs recovered from the soil of Greyhound farms in the USA were *T. canis* eggs (20). A recent survey of catteries in the Netherlands (18) showed a very low prevalence of *T. cati* in cats (2%) and no eggs were found in soil or dust samples.

From the results of this study and several other reports, it can be concluded that dog breeding kennels are an important source of *Toxocara* infection in dogs. In addition to the health implications for dogs with patent infections, one might consider also the health hazard to humans. The majority of breeders in our study (84%) kept their dogs in their own household environment, and even in their bedroom, during parturition and the first weeks after birth. However, surveys of the incidence of *Toxocara* infection of dog breeders and kennel workers show that the increased risk was high (2, 22) or that there was no increased risk (10, 13).

Without an adequate deworming scheme and use of appropriate anthelmintics, *Toxocara* eggs will be shed in the kennel environment and increase the infection pressure. Ultimately, infected pups will be sold to new owners and will represent a source of contamination of their new environment.

Nearly 72% of the dog breeders in this survey said that they gave anthelmintic treatment during oestrus and (or) pregnancy. Anthelmintics, at their recommended dosages, are not effective against inhibited somatic larvae, and treatment of bitches before mating and 2 weeks before anticipated whelping has no effect on prenatal transmission (8). Consequently, deworming pregnant dogs and cats is not recommended (4, 15). A lower percentage (63%) of the breeders dewormed their bitches post partum. Only 10 (31%) breeders who dewormed their dogs conformed to the recommended deworming schedule.

On the basis of these results, it is recommended that dog breeders should take more responsibility in the prevention of zoonotic infections, by implementing appropriate anthelmintic schedules and by using effective anthelmintics. In this way, breeders can provide parasite-free puppies and keep the kennel environment free of (zoonotic) parasites. An educational and information campaign on these topics would seem to be necessary.

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Chapter 5

A survey of *Toxocara* infections in cat breeding colonies in the Netherlands

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Abstract

Faecal samples from 225 adult cats and 112 kittens and dust and soil samples from 25 catteries in the Netherlands were examined for *Toxocara* eggs. The results of this survey showed a low nematode infection rate in the investigated Dutch catteries since only four adult cats (2%) from two catteries (8%) were found to shed *Toxocara cati* eggs. No other helminth eggs were seen in the faecal samples. Nematode eggs were not present in the environmental dust and soil samples from houses and kennels; only *Dipylidium caninum* eggs were found in only two samples of household dust.

Introduction

The intestinal helminths described in cats in the Netherlands include *Dipylidium caninum*, *Toxocara cati*, and *Toxascaris leonina* (3). *Toxocara* spp. infections have, because of their zoonotic significance, important public health consequences. Factors that have been shown to play a role in the epidemiology of *Toxocara* infections in humans in the Netherlands (2) include soil contamination (6), and infection of companion dogs and cats, stray cats (8), and dogs in breeding kennels (9). The present study was designed to determine the prevalence and potential risk factors of gastrointestinal nematodes, in particular *T. cati*, in catteries. The management practices of cat breeders were also investigated.

Material and methods

Sampling

Cat breeders were recruited via advertisements in cat breeding magazines. Twenty-five catteries, situated throughout the country, responded and were visited between March 1995 and October 1996. Faecal samples from 225 adult cats and 112 kittens were collected (cats younger than 6 months were considered as kittens). The cats were of 12 different breeds. In 24 of the 25 catteries the cats had access outdoors to gardens (8x) or to fenced kennels with concrete surfaces (16). Soil was sampled in seven catteries at three random locations using a garden trowel (± 500 g to a depth of 5 cm). Dust samples were taken from the floor, carpets, baskets, and cat sleeping places in 11 breeders homes (± 3 g) and from four kennels (± 31 g), using a hand-held vacuum cleaner. These samples were collected in 14 of the 25 catteries. Faecal, soil, and dust samples were stored at -20º C until examined. Data were gathered about

the animals, housing and nutrition, cleaning and disinfection procedures and the use of anthelmintics.

Techniques

Faecal examination was performed with a sedimentation-flotation technique, using a $ZnSO_4$ solution with a specific gravity of 1.3 g/cm³. The sensitivity of this test is 10 eggs per gram (8). Although this faecal flotation test is reliable for detection of eggs of helminths in general, the goal of our study was primarily to look for nematode eggs. Soil and dust samples were examined as described by Overgaauw and Boersema (9). *Toxocara* eggs in soil and dust samples were differentiated by the finer pitting of the protein layer of *T. cati* eggs as compared to that of *T. canis* eggs.

General description of the catteries

The average number of cats per cattery was 9 (range 2-20) with an average age of 4 years (range 2-8). In 72% (18/25) of the catteries, kittens (mean 8, range 1-14) with an average age of 11 weeks (range 3-26 weeks) were present. The mean number of litters per year was 4 (range 0.5 - 12). Eleven breeders (44%) claimed to maintain a closed colony; the others regularly introduced new cats. The floor of the indoor living areas or cat rooms/catteries included flagstones, wood surfaces, and linoleum. In four catteries there were also one or more carpets. All animals were kept in groups and, with one exception, the cats had access outdoors to fenced gardens (8x) or to concrete pens. Breeders cleaned the indoor living areas daily with a detergent. In addition, 40% (10/25) of the breeders used disinfectants on schedules ranging from once a week to once every 3 months. One breeder used a chlorine product, whereas the others used milder disinfectants. Two breeders (8%) confined their parturient queens in a fenced enclosure, and 11 (44%) confined them in the living room. The remaining 12 (48%) breeders confined parturient cats in their own bedroom for an average of 13 days after queening. All cats were fed on commercial dry or canned food. Seventeen breeders alternated this commercial food with cooked meat or fish. Adult cats were dewormed by 21 breeders (84%) 1.7 times (range 0 - 6) per year on average. Nine breeders (36%) gave an anthelmintic against nematodes to queens during oestrus or pregnancy and 11 (44%) also treated the queens post partum. Kittens were dewormed by 24 breeders (96%) 1.9 times (range 0 - 4) on average during the first 12 weeks of life. Three breeders (12%) complied with the recommended Netherlands deworming schedule by treating kittens at 4, 6, and 8 weeks of age (1). The average age of first kitten deworming was 7 weeks but in 35% (8/23) of kittens

deworming occurred at 8 weeks or later. The following active ingredients were used for deworming adult cats and kittens respectively: flubendazole (1x/2x), mebendazole (1x/0x), oxibendazole (6x/13x), piperazin (0x/1x), pyrantel (0x/1x), and pyrantel/febantel combination (13x/6x).

Results

Faecal samples

The results of the faecal examinations are given in Table 1. *T. cati* eggs were detected in samples from 4 of 225 adult cats (2%). These cats were in 2 of the 25 catteries (8%). In these catteries there were 6 and 11 adults cats and these cats were dewormed properly (3 to 6 times per year). One of these catteries had 1.5 litters per year and, when surveyed, there were 13 kittens, with an average age of 10 weeks. The other cattery had 6 kittens with an average age of 9 weeks. This cattery had the highest average number of litters (12 per year). All faecal samples (from 112 kittens) tested negative (e.p.g. <10). The mean age of the infected cats was 1.9 years (range 1 - 11) and all were female. Eggs of other nematodes were not detected.

parasite	**animals**	**samples positive (%)**	**catteries positive (n=25) (%)**
Toxocara cati	adult cats (n=225)	4 (2%)	2 (8%)
	kittens (n=112)	0 (0%)	0 (0%)

Table 1. Prevalence of Toxocara cati eggs in cat faeces and in cat breeding colonies

Soil and dust samples

None of the soil samples were positive for *Toxocara* eggs. *Dipylidum caninum* eggs were present in two household dust samples.

Risk factors

In view of the low prevalence of nematode infection, no significant correlation could be established between *T. cati* infection and epizootiological risk factors, such as number of adult cats or kittens, age of the kittens, litters per year, infected soil or dust, anthelmintic treatment schedules or active ingredients, disinfection procedures, or the feeding of uncooked meat.

Discussion

The role of *Toxocara cati* as a zoonotic parasite is not always recognized and the zoonotic risk for *T. cati* is considered to be less than that for *T. canis* (4, 5). This assumption was based on a survey in Iceland, where, to control hydatid disease, dogs have been banned since the Forties. While there is a large population of cats on Iceland, none of the 307 tested adult Icelanders were positive for *Toxocara* serum antibodies (13). Nothing was mentioned however about the cat population density, prevalence of *T. cati* in cats, the rate of environmental contamination, the expected survival rate of eggs in Icelandic soil and the assumed risk of soil contact by Icelanders. *T. cati* infection of various potential paratenic hosts, including chickens, mice, dogs and lambs, has been demonstrated (12) and man can also be considered as a potential host. Recently, it was suggested that *T. cati* can play an important role in human ocular larva migrans (11).

Cats may be infected with *T. cati* by transmammary infection, by eating paratenic hosts containing larvae, or by ingestion of infective eggs. There is no transplacental transfer of *T. cati*, unlike *T. canis* in the dog. Patent infections in kittens can first be seen at 6 weeks of age (10). Most domestic cats in the Netherlands go outdoors (8) and are likely therefore to be exposed to helminth eggs, particularly in areas with a high density of domestic or stray cats. Places used for defaecation are often shared by several cats. This may lead to contamination of cats' paws with infective eggs as they bury their faeces. Some cats hunt and eat birds and small mammals that can act as paratenic hosts for helminth parasites like *T. cati*. These risks were virtually absent for the breeding cats in this survey, since all outdoor areas were fenced and the soil in the gardens seemed not to be contaminated with helminth eggs. *T. cati* eggs were also not found in dust samples. Transmammary *Toxocara* infection seemed to be low in these breeding cats. As a consequence of the (irregular) deworming programmes and the limited access to outdoor environments and to wildlife, *Toxocara* did not seem to flourish in these cat breeding colonies. Nevertheless, the deworming schedules could be improved.

The prevalence of patent *T. cati* infections was low in the Dutch cat breeding colonies in this study. It should be considered that the experimental population in which the survey was conducted was a convenience sample of the reference population of Dutch cat breeders, because the breeders volunteered. This may also gave rise to biased results. Nevertheless,

different types of breeders and breeds from all over the country were included in the study. In the two catteries where adult cats were infected with *T. cati*, the presence of many older kittens, as well as the high breeding rate of one of the catteries, may be considered as contributing risk factors. Other reports in the literature of studies of cat breeding colonies were not found. Nichol et al. (7) reported on the prevalence of intestinal parasites in cats, using faecal samples obtained from veterinary surgeries and catteries, but did not distinguish between these two groups. They found *T. cati* (11.5%) and *T. leonina* (0.2%), with the highest incidence of *Toxocara* infections (up to 31%) occurring in animals younger than 6 months. The prevalence of 4.7% patent *T. cati* infections in cats from households and 21% in stray cats (8) and up to 15% *T. canis* in breeding dogs (9) in the Netherlands is considerably higher than that in the catteries in this study.

For animal and human health reasons it is necessary to prevent such infections and the consequent contamination of the environment. Prevention includes deworming with effective anthelmintics and using treatment schemes with emphasis on kittens. All faecal samples from kittens in this study were negative for *T. cati* eggs. This is surprising since only 44% (11/25) of these breeders dewormed their queens after parturition and not more than 12% of the breeders (3/25) applied the recommended deworming schedule. Moreover, 53% of breeders (8/15) applied the first anthelmintic treatment when kittens were older than 8 weeks and the first patent infections in kittens can be expected before this age. From these results, it can be concluded that *T. cati* infections are nearly absent in the investigated catteries and/or that the present measures are satisfactory despite deviations from the advised anthelmintic treatment schedule. Cat breeders should be provided with better information on these topics.

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Chapter 6

Incidence of patent *Toxocara* infection in bitches during the oestrous cycle

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Submitted

Abstract

The incidence of patent *Toxocara canis* infection as result of reactivation of somatic larvae with subsequent tracheal migration was investigated by faecal examination during 23 oestrous cycles of 15 bitches. Blood samples were collected for determination of total and differential leukocyte counts, prolactin concentration, and *Toxocara* titre. Five pregnant dogs were used as controls.

In the cyclic dogs there were no alterations in white blood cell counts or prolactin concentration, in contrast with the pregnant dogs, in which an elevation of both parameters was seen in the peripheral blood starting 10 days after onset of the luteal phase. The difference was significant at day 40 and day 60 (both $p<0.005$). No significant differences were observed in the number of eosinophils or in the *Toxocara* antibody titre. *T. canis* eggs were only found in the faeces of two 1-year-old, cyclic dogs at 60 and 140 days, respectively, after the onset of the luteal phase. It is concluded that cyclic beagle bitches, in which prolactin levels increase in the second half of the luteal phase, are unlikely to be at higher risk for patent *T. canis* infection.

Introduction

Infection with *Toxocara canis* in newborn puppies results from the tracheal migration of larvae which have arrived via the liver and lungs. The larvae break out into the alveoli and migrate through the trachea and pharynx. After being swallowed, they complete their development in the stomach and small intestine. Fewer newly hatched *T. canis* larvae develop into adult ascarids by the time the pup is two months old. This is due to acquisition of immunity against *T. canis* and the probability of somatic migration has increased progressively (23). After ingestion of infective *T. canis* eggs, adult dogs gradually accumulate somatic larvae in their tissues and the larvae persist for many years. During pregnancy, puppies are infected in the uterus from day 42 of gestation by somatic larvae that are reactivated within the tissue granuloma (9, 22). Although the initiating factors are unknown, hormonal influence during pregnancy has been indicated as a possible factor. Oshima (16) found that injections of prolactin led to a marked decrease in the number of *T. canis* larvae in tissues of mice that were experimentally infected and suggested that this hormone could be involved in stimulating dormant larvae to resume their migration.

Large and sustained doses of corticosteroids lower the resistance of otherwise resistant dogs and allow development of patent infection (10). The increased susceptibility to parasite infection in periparturient animals has also been explained by the 70 – 90% suppression of in vitro mitogen- and *T. canis* antigen-induced lymphocyte transformation (3, 12), and of the suppressed eosinophilia compared with non-productive controls at the same point of time (11). It was hypothesized that the immunosuppressive effect of pregnancy and lactation may permit tissue larvae or larvae from a newly acquired infection to initiate tracheal migration in the adult bitch and subsequent maturation in the intestine. This seemed to be supported by finding egg-producing *T. canis* in one postpartum bitch (11) at a time earlier than could be accounted for by ingestion of immature stages through ingestion of vomitus or faeces from her puppies (24).

Evans and others (5, 6) reported that the intact bitch during the luteal phase is at higher risk of a patent *Toxocara* infection. By faecal examination, three times as many bitches in the luteal phase were found to have patent *T. canis* infections than bitches in other groups. The findings were explained by the raising prolactine level in the cyclic bitch which reactivate dormant larvae to complete their cycle. Similar reports supporting this phenomenon were not found in literature.

In the present study, we report a comprehensive examination of such a possible reactivation of somatic *T. canis* larvae with subsequent tracheal migration in the cyclic bitch during the luteal phase and the relation of this phenomenon to plasma prolactin concentrations.

Material and methods

Animals

Fifteen beagle bitches, one to seven years of age, were used from November 1994 through August 1996. Data from 23 oestrous cycles of these bitches were collected. Dogs were housed singly or in pairs in indoor-outdoor runs with concrete floors. Sometimes the dogs had access to grass playing grounds. An ovoid box of synthetic material was available as a bed. A commercial dry canine diet was supplied once daily and water was available ad libitum. The dogs were checked three times weekly for the presence of vulvar swelling and sanguineous vaginal discharge, indicating the onset of pro-oestrous. Pseudopregnancy was not observed. During the investigation period, an anthelmintic was given to individual dogs only if eggs of intestinal nematodes were found in the faeces. *Toxocara* infection was present in the colony,

as proved by the expulsed worms after deworming in every litter born during the last few years. The five pregnant dogs used as controls consisted of two beagles, two and three years old, belonging to the same colony; a three- year-old Great Dane, a two-year-old Labrador retriever, and a three-year-old Drentse patrijs. It was the first parity for all dogs.

Sampling

From the onset of pro-oestrous, blood samples were collected by jugular venipuncture into EDTA tubes. The samples were centrifuged for 10 minutes at 2000 g and the plasma was stored at –20º C until progesterone, prolactin, and *Toxocara* antibody titre were measured. For determination of the progesterone concentration, blood samples were collected on the day of onset and subsequently three times per week. Day 1 of the luteal phase was defined as the first day after onset of the follicular phase on which the progesterone concentration in the peripheral blood had reached 16 nmol/l (2, 13).

Blood samples were collected for haematological examination and determination of plasma prolactin concentrations in the cyclic dogs at day 1, 10, 20, 30, 40, 60, 80, and 140 after the onset of the luteal phase, and in the pregnant dogs on day 1, 10, 20, 30, 40, and 60 after the onset of the luteal phase. Haematological examination was also performed on day 10 post-partum.

For determination of *Toxocara* antibody titre blood was collected from the cyclic dogs on days 1, 40, 60, 80, and 140 after the onset of the luteal phase and from the pregnant dogs on days 1, 40, and 60 after the onset of the luteal phase and on day 10 post-partum.

Faecal samples for parasitological examination were collected from the cyclic dogs on days 1, 40, 50, 60, 70, 80, and 140 after the onset of the luteal phase and from the two pregnant beagles on days 1, 40, 50, and 60 after the onset of the luteal phase and on days 6, 10, 14, and 18 after parturition together with faecal samples from their puppies.

Methods

Plasma concentrations of prolactin were determined by a previously validated heterologous radioimmunoassay (13). The intra-assay and interassay coefficients of variation were and 3.5% and 11.8%, respectively. The lowest detectable amount of prolactin was 0.8 µg/l plasma.

Plasma concentrations of progesterone were determined by a previously validated heterologous radioimmunoassay (4, 13). The intra-assay and interassay coefficients of

variation were 11% and 14% (n=12), respectively, and the limit of quantification was 0.13 nmol/l.

Haematological examination consisted of total white blood cell (WBC) counts determinated by a Helios Haematology Analyser (ABX, France), and differential leukocyte counts based on 100 cells in May-Grünwald Giemsa stained blood smears.

Toxocara plasma antibody titres were determined with an ELISA (8) validated for the dog using *Toxocara* excretory-secretory *(*TES) antigens (19). The lowest detectable titre was < 1:10. Parasite-free dogs from a specific pathogen free beagle colony (Harlan, Zeist NL) were used as negative control.

Faecal examination was performed with a sedimentation-floatation technique using a $ZnSO_4$ solution with a specific gravity of 1.3 g/cm^3 (17). The lowest detectable number was 10 eggs per gram (epg).

Data analysis

The Mann-Whitney U test, in Solo 60.4 (BMDP Statistical Software, Delta Soft), was used to compare the means, applying a significance level ≤ 5%. A log transformation was performed before analysis of the *Toxocara* titre data. When appropriate the paired *t* test (two-tailed) was used to compare the means. The values were expressed as means ± sd.

Results

The mean total number of white blood cells of the cyclic dogs did not vary markedly, in contrast to the pregnant group, in which an increase was seen from day 10 (Figure 1). Increasing neutrophils were responsible for this leukocyte rise and the values exceeded the upper limits (reference value: 5.9 – 13.8 x 10^9/l) during the second half of the pregnancy. At 10 days post-partum, the value was 13.8 ± 1.5 x 10^9/l. The difference from the cyclic dogs was significant on day 40 ($p<0.002$) and day 60 ($p<0.001$), respectively.

The cyclic dogs did not show important changes in the absolute number of eosinophils in the blood. An increase in eosinophils was apparent in three of the five pregnant dogs from day 20 (Figure 2). The mean number of eosinophils in the pregnant dogs was considerably higher and exceeded the upper limits several times (reference value: 0.10 – 1.25 x 10^9/l). The difference between the two groups was not significant.

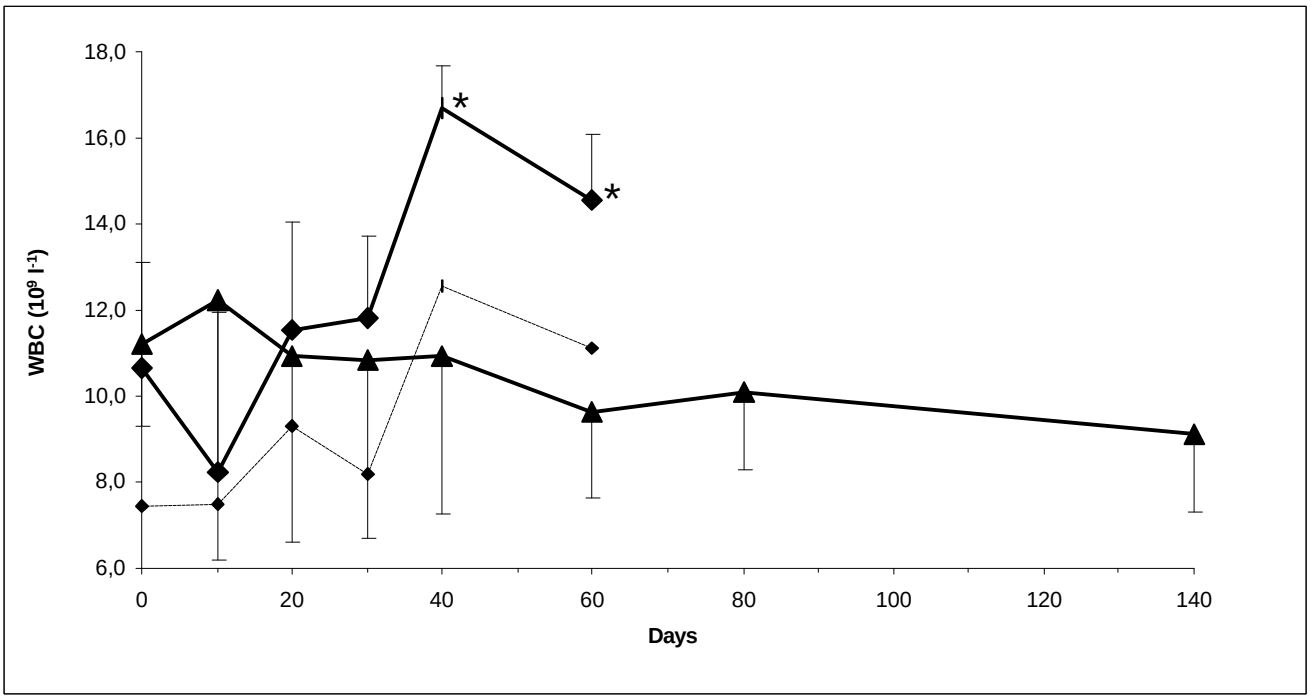

Fig. 1. Mean ± sd absolute number of leukocytes (—) and neutrophils (·····) in the blood of 23 cyclic bitches (▲) and 5 pregnant bitches (◆). D0 is the onset of the luteal phase. (* : significant difference).

The average of the mean plasma prolactin concentration (4.5 µg/l; s.d 1.5) in the cyclic dogs during the first half of the luteal phase (day 1 – day 40) was significantly different (p=0.003) from the average of the mean plasma prolactin concentration (10.0 µg/l; s.d. 8.5) during the second half of the luteal phase (day 41 - day 80). In all pregnant dogs the mean prolactin concentration increased from day 10 to a maximum of 120 ng/ml on day 60 (40.8 ± 50.3 µg/l during the entire experiment). The observed differences between pregnant and cyclic dogs were significant on day 40 (p=0.0048) and day 60 (p=0.0007), respectively.

The mode of the ELISA *Toxocara* titres in the cyclic dogs increased from 1:40 between day 0 and day 80 to 1:80 on day 140 (Figure 4). The individual titres of 16 dogs were stable or changed slightly. In four animals the titre increased and in three it decreased by more than one dilution step. The titre of one dog with *T. canis* infection increased from 1:20 on day 40 to 1:320 on day 60. The mode of *Toxocara* antibody titres in the pregnant dogs increased from 1:20 to 1:160 by day 40, but differed among the dogs. The titre in one dog decreased from 1:640 on day 0 to 1:10 on day 60; the titres in three other dogs increased by no more than one step of dilution and in one dog the titre did not change.

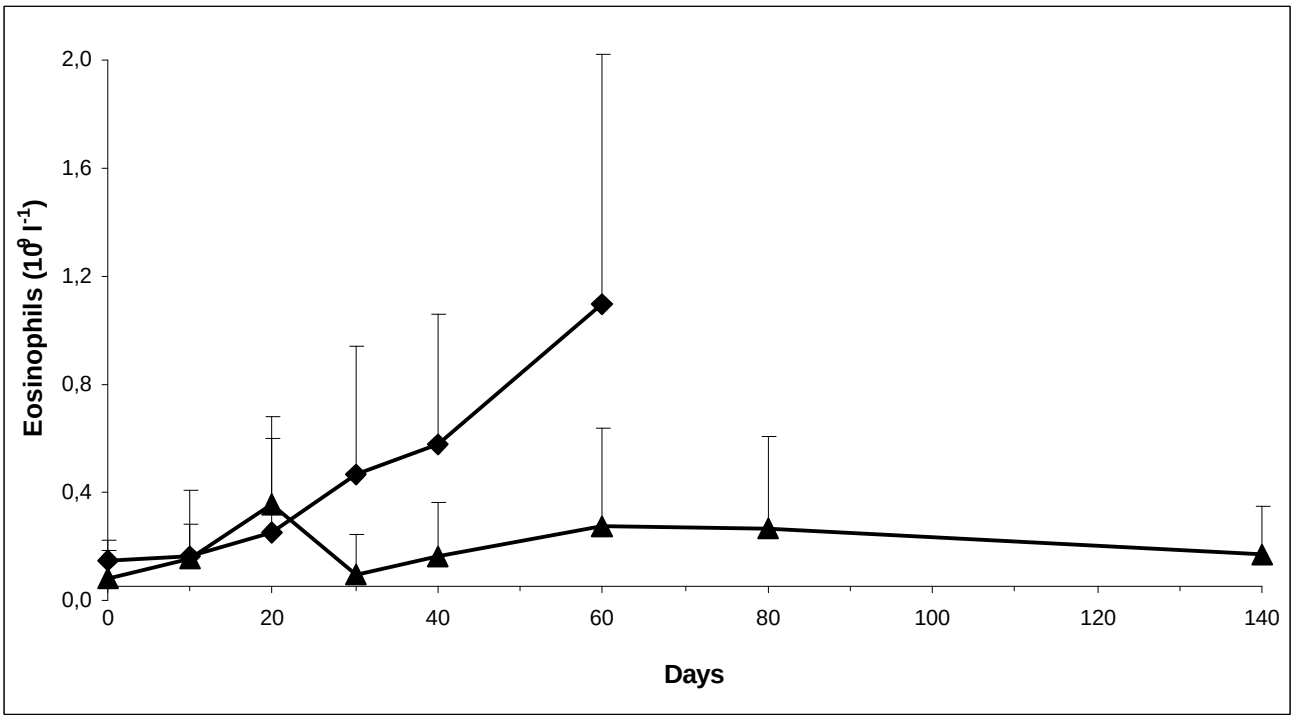

Fig. 2. Mean ± sd absolute number of eosinophils in the blood of 23 cyclic dogs (▲) and 5 pregnant dogs (◆). D0 is the onset of the luteal phase.

In faecal samples of two one-year-old cyclic dogs, *T. canis* eggs were detected on day 60 (epg 50) and day 140 (epg 560), respectively. In a faecal sample of a one-year-old cyclic dog *T. canis* eggs were found on day 0 (epg 180). This dog was treated and remained negative. *Trichuris vulpis* eggs were found in faeces of 6 other cyclic dogs at different intervals (days 0, 40, 60, and 80). One two-year-old cyclic dog had *Toxascaris leonina* eggs in the faeces on day 140 (epg 10).

Faecal samples from the two pregnant beagle dogs were examined up to 18 days after birth and all were found to be negative for nematode eggs. In the litters of these beagles, *T. canis* eggs were shed by their puppies from 3 weeks of age.

Discussion

Toxocara infections of dogs and cats are important zoonoses. Prevention consists in general of hygienic measures, elimination of the parasite from pets by anthelmintic treatment, and by directing information and education to increase awareness of the potential zoonotic hazards (20).

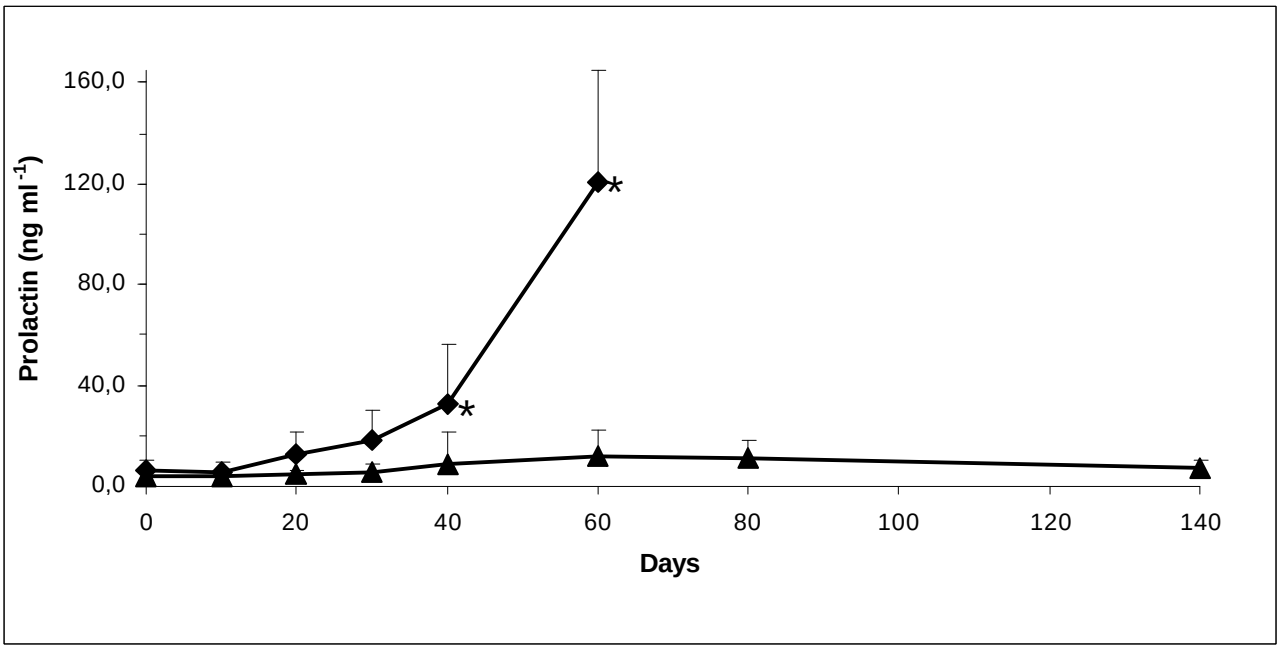

Fig. 3. Mean ± sd plasma concentrations of prolactin of 23 cyclic bitches (▲) and 5 pregnant bitches (◆). D0 is the onset of the luteal phase (* : significant difference).

Evans (5) performed a cross-sectional pilot survey in which 181 bitches were examined for the presence of *Toxocara* ascarids during routine visits to veterinary surgeries. Of the dogs in metoestrous (described as 30-60 days post oestrous), 17% had a patent *T. canis* infection, whereas the prevalence in other groups (pregnant, spayed, in anoestrous, or ovulation suppressed by medication) was 5%.

Nothing was mentioned about the history of the dogs and only animals that had been dewormed in the preceding four weeks were excluded from this experiment. Such a study design provides an impression about the frequency of disease (patent *T. canis* infection) or of the factor (period of the oestrous cycle). It is difficult to extrapolate the results to population values because *T. canis* prevalence in adult dogs is in general low (1, 17) and large numbers of samples are required. Furthermore, it is not known how closely the distribution of some risk factors or the prevalence found in the discussed study might be to dog population values. The author hypothesized that a rise in prolactin from the 40th day after the onset of the luteal phase is responsible for the activation of somatic larvae in the cyclic bitches.

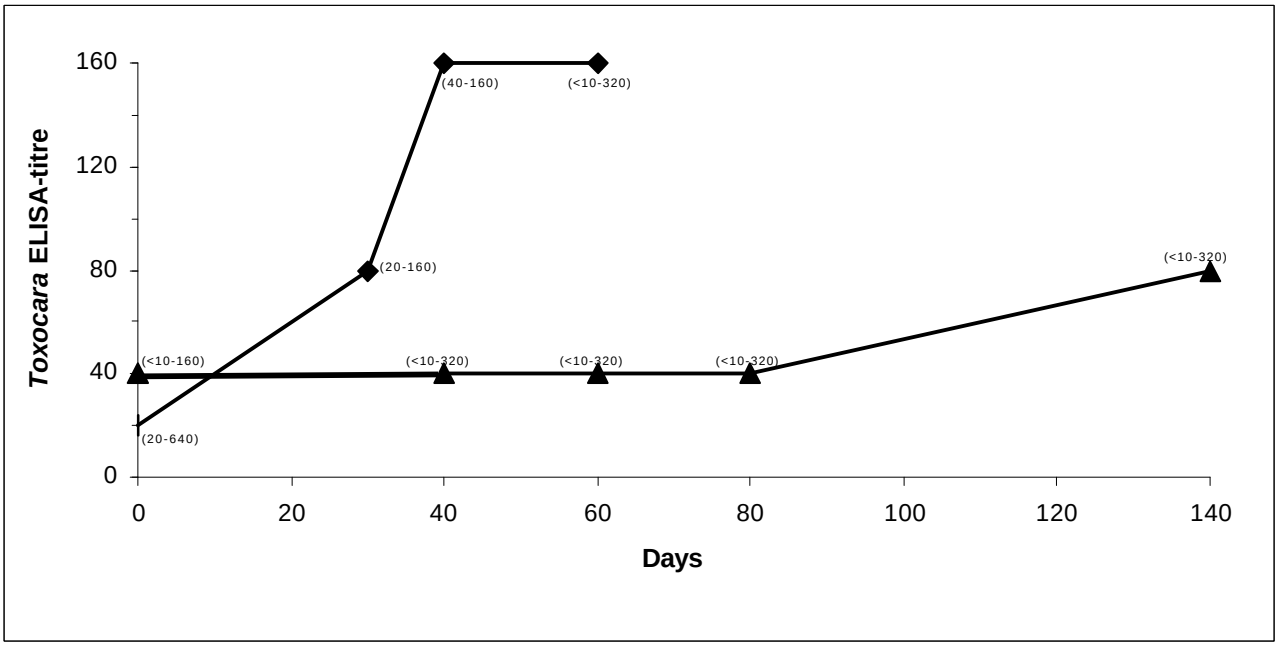

Fig. 4. Mode (and range) of the plasma Toxocara ELISA titre of 23 cyclic bitches (▲) and 5 pregnant bitches (◆). D0 is the onset of the luteal phase.

However, since there is a delay of 4 to 5 weeks before eggs appear in the faeces after tracheal migration (7, 18), the first *Toxocara* eggs cannot be expected in the faeces before day 75. This is considerably longer than the luteal phase.

Our study revealed a slight but significant increase in prolactin concentration in cyclic beagle dogs, but the absolute values remained low in contrast to pregnant dogs. An increasing prolactin level was not found in a previous report (14). A higher *Toxocara* infection rate was not found during the luteal phase. Only one young cyclic dog shed *T. canis* eggs at the end of the luteal phase (day 60) and another dog at a much later moment (day 140). This finding in combination with the occurance of infected litters confirmed at least the infected status of the beagle colony with this parasite and are most probably the result of environmental infection in line with the *T. vulpis* and *T. leonina* infections. The eosinophilia in the peripheral blood of the pregnant dogs in our study may be an indication of reactivation of somatic larvae and subsequent migration from day 42 of the gestation, since eosinophilia was not observed in uninfected parturient bitches (12). In contrast to this report an increase in the total number of white blood cells occurred in our study and a periparturient suppression of the eosinophils was not seen. The occurrence of reactivated *T. canis* larvae was not supported by marked elevation of *Toxocara* titres. There was a slight increase in the mode titre in the pregnant

dogs, but in one of these dogs the titre remained stable and in another it decreased. On the other hand, a decrease can be explained by an excess of *Toxocara* larvae antigen that binds circulating antibodies. The *Toxocara* ELISA test may show markedly increasing antibody titres to TES antigens in dogs after experimental infection with large amounts (10.000) of *T. canis* eggs (7, 21, 25). It may therefore be possible that only massive (experimental) *Toxocara* infections will result in important alterations in the *Toxocara* titre. Under natural conditions, the low numbers of migrating larvae may not induce a marked increase in the *Toxocara* titre. This is in accordance with the observation that only half of the puppies (4/8) had a detectable antibody titre 5 weeks after experimental inoculation of lower numbers (100 eggs) of *Toxocara* eggs (7).

The moderate alterations in the *Toxocara* titres of some cyclic dogs in our study may be an indication of the existence of a stimulus by *T. canis* larvae during the investigation period. Differences in eosinophil counts, however, did not occur in this group. Taking also into account the faecal examination results, it seems that cyclic bitches are not at higher risk for patent *T. canis* infection. It is, however, questionable whether pseudopregnant bitches, which show a marked elevation in the prolactin concentration (15) will develop patent *T. canis* infections.

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Chapter 7

Anthelmintic efficacy of oxibendazole against some important nematodes in dogs and cats

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Abstract

The anthelmintic efficacy and safety of the oxibendazole component in a combination oxibendazole-niclosamide paste were investigated in dogs and cats and in litters of pups with naturally acquired nematode infections. A single dose of 15 mg oxibendazole/kg body weight given to 70 dogs and to 29 cats reduced faecal worm egg counts (EPG) by 97.6% for *Toxocara canis*, 95.7% for *Trichuris vulpis*, 94.6% for *Ancylostoma caninum* and 100% for *Toxascaris leonina*. In cats, 96.7% efficacy was demonstrated against *Toxocara cati*. In a second trial, 119 pups in 22 litters were treated with the same dosage at 2, 4 and 6 weeks of age. After treatment on two consecutive days, 95% of the pups did not shed *T. canis* eggs, compared with 85% after only a single treatment. Side effects were rare and only recorded in young animals. A two day treatment schedule is recommended for unweaned pups.

Introduction

In spite of the availability of numerous anthelmintics, intestinal nematode infections of dogs and cats continue to be a significant problem, particularly in relation to the zoonotic significance of *Toxocara* ascarids and *Ancylostoma* hookworms. Anthelmintic strategies, consisting of a combination of treatment schedules and the choice of appropriate anthelmintics, are therefore considered to be important and deworming schedules directed against such parasites have been published (4). The purpose of an anthelmintic treatment directed against gastrointestinal nematodes is to stop egg output and to expel worms from the pet. Due to a poor solubility in gastro-intestinal secretions, benzimidazoles have minimal gastrointestinal absorption except for thiabendazole, albendazole and oxfendazole (20). They have significant larvicidal effects (1, 13) and are virtually without toxicity at therapeutic dose rates (7, 9). Oxibendazole (methyl 5-n-propoxy-2-benzimidazole-carbamate; OBZ), was first synthesised in 1973 (24). It is used against many endoparasites in a wide range of hosts. Toxicology studies in companion animals support a wide margin of safety for OBZ (2, 3). Attempts to determine an LD50 for OBZ in small laboratory animals failed because rats and mice tolerated the maximum quantities (10,000 mg/kg) that could be physically given (20). Oxibendazole is indicated for several nematodes of dogs and cats, such as *Toxocara*, *Toxascaris*, *Trichuris*, *Ancylostoma* and *Uncinaria* (6, 8). The efficacy of OBZ against endoparasites in dogs and cats after single administration, however, has been little

investigated, in contrast to larger animals such as cattle, sheep and horses (7, 20, 21). This report describes trials in dogs and cats to investigate the anthelmintic efficacy of OBZ, administered in a combination with niclosamide, based on faecal egg count reduction. Co-incidental observations relating to treatment safety were made. Niclosamide is a taeniacidal drug and not reported to have any action against nematodes (20).

Material and methods

Animals

Trial 1. In the period between 1993 and 1996, 55 naturally infected dogs (23 male and 32 female, average age 2.1 years, range 0.1 - 7 years) and 28 cats (15 male and 13 female, average age 2.5 years, range 0.1 - 11 years) were used in this trial, conducted in the Netherlands. The animals were of different breeds and body weights, and located at veterinary practices, breeder kennels, catteries and in a colony of purpose bred beagles. Eleven pups from Surinam with *A. caninum* infections (6 male and 5 female, average age 7 weeks, range 4 - 10 weeks) were included since hookworm infections are not common in the Netherlands (19). All dogs and cats were healthy.

Trial 2. A second group consisted of 119 newborn pups in 22 litters (20 pure bred and 2 mixed breed) at breeders in different locations in the Netherlands.

Drug formulation and treatment

Because OBZ is not licensed as a single active ingredient for pets, a registered combination paste of 3% oxibendazole and 24% niclosamide (Vitaminthe®, Virbac Laboratories SA, France) was used.

The animals in both trials were treated at a dose rate of 15 mg OBZ/kg body weight, corresponding to 1 ml of paste/2 kg b.w., applied on the back of the tongue. In *trial 1* only a single dose was administered.

The pups of *trial 2* were treated, either once or twice on consecutive days, when 2, 4 and 6 weeks old (3 or 6 treatments in total, respectivily). The anthelmintic was administered by either a veterinarian or the owner and the treated animals were fed ad libitum before and during treatment.

Faecal examination

Faecal samples from animals of *trial 1* were taken 1-3 days before and 7 days after treatment and in *trial 2* only at 7 days after each treatment (sampling at 3, 5 and 7 weeks of age). A first pre-treatment faecal examination at 2 weeks of age was not performed, because prenatally infected puppies may be passing eggs in the faeces not earlier than by 3 weeks of age (22).

The faeces were stored in plastic tubes at -20° C until examined. Faecal worm egg counts (eggs per gram; EPG) were performed as described by Overgaauw (19). Species differentiation of hookworm eggs was performed by measuring dimensions.

Efficacy evaluation

Efficacy calculations were based both on faecal egg count reduction and by calculating the percentage of animals with negative (EPG <10) egg counts after treatment. Individual animals served as their own controls.

An average percentage reduction in mean egg counts of 90% or more was considered effective, 80-89% moderately effective and 60-79% low efficacy. Values below 60% were considered ineffective (14).

Palatability and side effects

After each treatment, the animals were observed to evaluate whether the paste formulation was palatable. The animals were monitored by the owner/attendant for general behaviour, appetite and excretions.

Statistical analysis

The significance of the difference in mean egg counts in the 2 groups of pups was determined by 'Pearson's Chi Square', in Statistix 3.5 (NH Analytical Software, St. Paul MN), applying a significance level ≤ 5%.

Results

Efficacy in dogs in trial 1

The results of the mean egg counts in dogs are shown in Table 1. Single treatment reduced mean egg counts of ascarids (*Toxocara, Toxascaris*), hookworms (*Ancylostoma*) and whipworms (*Trichuris*) by between 94.6% and 100%. All hookworms were determined as *A. caninum.* Five of 11 dogs infected with this parasite were still positive, but with a

considerably lower egg count. These dogs were younger than 3 months. Four dogs were infected with both *Ancylostoma caninum* and *Toxocara canis.* After deworming, they were all negative for *T. canis,* but only pup negative for *A. caninum* as well. The 6 dogs that were still shedding *T. canis* eggs after deworming had an average age of 11 months while the 5 remaining dogs with persistent *Trichuris vulpis* eggs were 4 years old. *Toxascaris leonina* was the most susceptible worm species since the treatment was 100% effective.

parasite	**no. negative/ no. treated**	**mean epg**		**EPG reduction**
		before treatment	**after treatment**	
Ancylostoma caninum	6/11	1246	67	94.6%
Toxascaris leonina	4/4	745	0	100%
Toxocara canis	22/28	889	21	97.6%
Trichuris vulpis	22/27	699	30	95.7%

Table 1. Results of a single treatment of 15 mg oxibendazole/kg b.w. against some nematodes in dogs.

Efficacy in cats in trial 1

The results of the mean egg counts in cats are shown in Table 2. One cat was infected both with *T. leonina* and *T. cati*, all others were infected only with *T. cati*. Substantial reductions in *T. cati* egg counts were observed, although five of the 28 cats remained positive (all single infections). The average age was 2 years.

parasite	**no. negative/ no. treated**	**mean epg**		**epg reduction**
		before treatment	**after treatment**	
Toxascaris leonina	1/1	30	0	100%
Toxocara cati	23/28	1137	38	96.7%

Table 2. Results of a single treatment of 15 mg oxibendazole/kg b.w. against some nematodes in cats.

Efficacy in puppies in trial 2

The results of treatment of the 119 pups are shown in Table 3. It was not possible to collect faecal samples from every pup every two weeks, since some pups had already been delivered to the new owner at 7 weeks of age. Until 7 weeks of age, eighty-five percent of the pups that received a single treatment at 2, 4 and 6 weeks did not shed *T. canis* eggs after treatment, while ninety-five percent of the pups treated on 2 successive days were cleared during this period. The difference (i.e. number of pups negative) was significant in pups sampled at 5 weeks of age ($\chi^2 = 4.5$ and $p < 0.05$) and also comparing single and double treatment values ($\chi^2 = 7.0$ and $p < 0.01$).

	3 weeks of age		**5 weeks of age**		**7 weeks of age**		
Treatments	**no. negative/ no. treated**	**mean EPG**	**no. negative**	**mean EPG**	**no. negative**	**mean EPG**	**total**
1 treatment	40/49	242	31/40	50	54/58	14	125/147 (85%)
2 treatments	48/53	13	26/27	7	51/52	2	125/132 (95%)
p-value ($\chi^2_{df=1}$)	*0,19*		*0,03**		*0,21*		*0,008**

* *significant difference*

Table 3. Results of a single or double treatment of 15 mg oxibendazole/kg b.w. against some nematodes in unweaned puppies

Palatability and side effects

Information was recorded from 55 animals in *trial 1* (23 dogs and 32 cats). Two 8 weeks old cats spat out the paste. One 10-week-old cat was described as sleepy and one 16-week-old dog and two 8 week old cats vomited after treatment. In *trial 2*, five Dachshund pups in the same litter showed persistent mild diarrhoea throughout the study, although the relationship to treatment was uncertain.

Discussion

The results demonstrated a mean egg count reduction for ascarids, whipworms and hookworms of between 95% and 100% after treatment with the oxibendazole-niclosamide paste. Niclosamide was not considered to have contributed to efficacy since it is a taeniacidal drug and has not reported as having an action against nematodes (20) and OBZ was therefore

considered the active agent. Except for the hookworm infections (all in young pups), the faeces of more than 80% of the animals were free of nematode eggs after treatment, while the others had considerably diminished egg counts. Further research may be merited to investigate efficacy after double treatment on 2 consecutive days in older animals.

The results of the egg counts in pups in *trial 2* showed that considerably more pups were cleared after treatment on two consecutive days (91% - 98%) than after single treatment (78% - 91%). A better efficacy after repeated administration can be explained by the relatively short gastrointestinal tract of carnivores with a short transit time as result.

Occasional and rare diarrhoea and vomiting in our study may be attributed to the niclosamide component (20). Lower efficacy values can be a consequence of diarrhoea since this will alter the rate of passage of the anthelmintic (15). Diarrhoea can be exacerbated by physiological instabilities associated with the stress of weaning, change of diet and being moved to a new environment (12).

Oxibendazole (in combination with diethylcarbamazine) at the low daily dosage of 5 mg/kg continued for at least 7 days, has been shown to be effective against *T. canis*, *T. leonina*, *T. vulpis*, *Uncinaria stenocephala* and *A. caninum* infections. Several groups of authors confirmed this efficacy: McCall et al. (18) in a double-blind controlled critical challenge study, Stromberg et al. (23) in a critical study with naturally infected pups and Armstrong et al. (5) and Marley et al. (17) in clinical trials where pups were included. Fenbendazole administered in comparable schedules with treatments of 50 mg/kg on three consecutive days to unweaned puppies also reduced the overall numbers of *T. canis* by 90% to 94% (9) and, compared with untreated control pups, reduced *T. canis* egg output by 88% - 95% after a single treatment of 100 mg/kg (10). A single treatment with a combination of febantel, pyrantel and praziquantel induced 85% reduction in egg-output when given to pups at 2, 4 and 6 weeks (10).

The risk of toxocarosis in man and animals can be minimised by reducing contamination of the environment with *Toxocara* eggs. This should be a primary objective of any control programme (13). Efficacy of anthelmintics for dogs and cats is often evaluated only in adolescent or adult dogs (11), although nursing pups and kittens are more frequently and heavily infected and hence present the greatest potential risk to the environment and trials on them should be included if possible. The population dynamics of *Toxocara* in unweaned puppies (somatic migration of larvae at the moment of deworming and re-infection throughout the suckling period) require repeat dosing of anthelmintics after 10 to 14 days. Efficacy of anthelmintics can be influenced by diarrhoea (increased intestinal passage) and

the fat contents of the bitches' milk (16, 13). Treatment efficacy should be measured in several geographical locations in order to address variability in environmental conditions and possible worm strain variations (14).

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Chapter 8

Effect of a government education campaign in the Netherlands on awareness of *Toxocara* and toxocarosis.

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Abstract

An education campaign in the Netherlands on the dangers of *Toxocara* spp. was performed by the Dutch Ministry of Public Health in 1993, consisting of sending information to veterinarians and physicians, publications in medical journals and popular magazines, brochures, and information on radio and television. Before and after the campaign, respectivily, surveys were performed among 200 and 105 veterinarians, 135 and 105 physicians, 511 and 530 pet owners and 100 and 261 non-pet owners to investigate their knowledge and perception concerning *Toxocara* and toxocarosis. The knowledge of this condition before the campaign was inadequate in veterinarians and physicians and generally absent in the public. After the extensive informational campaign, the knowledge of veterinarians was increased for several topics but still inadequate, and awareness had not changed for physicians and the public. For veterinarians and physicians, prolongation of education concerning these subjects is recommend; for physicians and pet owners better ways to provide information are needed.

Introduction

Toxocara roundworms are probably the most common gastrointestinal helminths of the domestic canids and felids. Infection can occur by ingestion of infective eggs, intra-uterine with larvae (only dogs), by ingestion of larvae with colostrum and milk and by ingestion of infected paratenic hosts. Infection with adult *Toxocara* spp. is mainly seen in young animals, while infection of adult animals often results in inhibited somatic larvae. In dogs, adult egg-producing *T. canis* can be found at 2 to 3 weeks of age and in kittens the first eggs of *T. cati* at 7 weeks of age (13, 17). The clinical signs depend on the age of the animal and on the number, location and stage of development of the worms but are rare in adult dogs and cats. Puppies can suffer from pneumonia, emaciation and digestive disturbances because of the tracheal migration and death can occur. Recent studies revealed a prevalence of *Toxocara* in adult dogs and cats in Dutch households, not higher than 5% (12).

Toxocarosis also is a zoonotic disease. After ingestion of infective *Toxocara* spp. eggs by humans, *Toxocara* larvae may migrate through the body, resulting in somatic larvae in many types of tissues, visceral larva migrans or VLM (8, 4). The multisystem invasion can be

associated with varied nonspecific clinical symptoms as a result of the host's immune response (5). Fatal cases are rare. If subsequent exposure to *Toxocara* eggs is avoided, the disease is usually self limiting (7). Ocular Larva Migrans (OLM) is characterised by complaints of loss of visual acuity, squint and 'seeing lights'. In a minority of cases, total blindness of one or two eyes can resulted (6). A commonly found clinical syndrome in OLM is a posterior pole granuloma mimicking a retinoblastoma. The exposure to *Toxocara* spp. in the Netherlands, based on serologic surveys, is estimated by Buijs (2) between 7% in children and 20% in adults. OLM cases in the Netherlands over the last decade did not exceed 10 cases (annual incidence 1:15 million) as estimated by the the Netherlands Opthalmic Research Institute, National Uveitis Workshop (Personal communication Prof. Dr. A. Kijlstra, Chairman, 1993).

The prevention of infection of humans with embryonated *Toxocara* eggs is based on three general principles: prevention of environmental contamination, hygiene of children and adults, and education of the public (particularly pet owners) about public health risks and the social concepts of responsible pet ownership (15). The most important part of prevention of environmental contamination is a recommended anthelmintic schedule in puppies, kittens, nursing bitches and queens and routine deworming of adults. Anthelmintics are in the recommended dosages not effective against inhibited somatic larvae and it is therefore not advised to deworm pregnant dogs and cats.

In 1993 the Dutch Ministry of Welfare, Public Health and Sport, Department of Veterinary Inspection, decided to start an informative campaign about *Toxocara* and toxocarosis for the Dutch population with as objective a measurable improvement of knowledge. Previously, the close related professions of veterinarians and physicians were informed about the campaign and updated with knowledge of these topics. After informing the veterinarians and physicians, a 100% knowledge of *Toxocara* and toxocarosis or awareness were to find this information (e.g. education campaign), was expected. Nothing was known sofar about the actual knowledge.

In this study the goal was to estimate the effect of the campaign by evaluation the knowledge, recommendations and attitudes of the veterinary and medical professions as well as the knowledge of the public, by performing surveys before and after the education campaign.

Material and methods

The education campaign

The education campaign was carried out in 1993 and prepared, coordinated and supervised by a commission formed by specialists of the Dutch Veterinary Inspectorate (Ministry of Welfare, Public Health and Sport, The Hague), National Institute of Public Health and the Environment (Bilthoven), Faculty of Veterinary Medicine (University of Utrecht), Dutch Foundation of Pet Animals and the pharmaceutical industry.

Each Dutch veterinarian and physician was informed in April 1993 by mailing an information pack containing: 1. personalised introductory letter, 2. overview of the campaign, 3. brochures to place at clients disposal in the waiting room or for distribution and 4. articles written by specialists titled a) '*Toxocara* species, undesirable guests', b) 'Sandpits and health' and c) 'Roundworms, risks for health of man and animal' (*articles available on request*).

In May 1993 article (a) was published (1). Also in May articles (b) and (c) were published in weekly magazines that were delivered free of charge to all Dutch households. At the end of May, publication (a) was published in general dog/cat magazines and magazines for dog-fanciers. In August, an article written by a physician (16) was published in the Dutch Journal for Physicians. An unlimited supply of free brochures for clients could be ordered by veterinarians and physicians. On television, the risk of *Toxocara* for man and the importance of deworming animals was discussed in a popular animal series early in the evening designed to reach children. In May and June 1993, radio commercials were broadcast several times on 4 national radio stations.

Surveyed groups

Measuring a difference of 20% with a power of 80% and a confidence of 95%, the required sample size for veterinarians and physicians was calculated as 100 and with a power of 90% for the households as 500 (3). Since the fraction correct answers was previously not known and could vary per question and a certain non-response percentage should be taken into account, a larger sample was used. A computer generated from files of all Dutch practicing veterinarians (n = 2213) and physicians (n = 6595) at random 210 (9.5% sample), respectivily 226 (3.4% sample) telephone numbers. A random computer sample of 611 adresses of households (including 100 non-pet owners), equally divided over the region of the city of Amersfoort, was obtained from a commercial agency. One year after the education campaign in a similar way, the phone numbers of a different group of 111 veterinarians (4.9% sample) and 150 physicians (2.3% sample) and adresses of 781 households (including 261 non-pet owners) in the region of the city of Apeldoorn were selected. Amersfoort and Apeldoorn were

selected because these areas are considered by Dutch market research agencies as representative for the Dutch population concerning socio-demographic properties (personal communication Marketresponse BV Amersfoort).

Data collection

The first study was carried out as anonymous telephone-administered interviews of *veterinarians* (July and August 1992) and *physicians* (February 1993). Only one practitioner per practice was interviewed by a person without a biomedical education. Both second surveys were performed during November 1993 until February 1994.

The *pet-owners and non pet-owners* were visited by 4 students from the School of Economics and Management Utrecht, during January and February 1993. The second interview took place in the period September - December 1993.

To keep the answers clear and short, 'closed questions' were used as much as possible. In relevant questions the answers were expressed in percentages.

Questionaires (available on request)

Data collected from *veterinarians* include general information of the veterinarian (sex, year of graduation) and practice (location, companion/mixed/large animal, partnership), anthelmintic strategy in dogs and cats (age first deworming puppies and kittens, interval, routine deworming adult animals) and public health aspects (zoonotic risks, estimated infection rate of puppies, estimated infection risk of children in direct contact with puppies and kittens, the need of informing pet owners about deworming).

The questionaire for *physicians* included general information of the respondent (sex, year of graduation, university of graduation) and practice (location, partnership) and public health aspects (zoonotic risks, estimated infection rate of puppies, estimated infection risk of children in direct contact with puppies and kittens, the need of informing the public about zoonotic risks).

The questions for *pet owners* involved general information regarding household and animal(s), last anthelmintic treatment of their pets and zoonotic risks. *Non pet-owners* were asked about general information of the household and knowledge about worms and zoonotic risks due to pets.

All questionaires used in the second survey were expanded with a question about notification of the education campaign; the public was also asked via which channels the information was

obtained. For pet-owners, a question was added about whether notification of the education campaign led to deworming and who actually advised deworming.

Measurement of effect

To measure the knowledge of the veterinarians and physicians, some unequivocal topics were questioned. The correct answers before and after the campaign can be compared and evaluated statistically. The following topics were selected for this purpose.

* *Age of first anthelmintic treatment* (veterinarians). The advised first deworming for puppies should be at 14 days after birth. A tolerance of +/- 4 days was considered acceptable.
  The first deworming of kittens should take place at 6 weeks of age at the latest. Since the Dutch Ministry of Public Health advised, however, a first deworming age of 4 weeks, the acceptable range was considered between 21 to 49 days.
* *Interval of deworming* (veterinarians). Treatment should be repeated every 2 weeks prior to developing of new arrived larvae. Only data from the most important period, up to 12 weeks post partum, were used with an acceptable range between 10 to 18 days.
* *Zoonotic risks of Toxocara* (veterinarians and physicians). This is without any doubt proved for as well *T. canis* as *T. cati*.
* *Infestation rate of newborn puppies with Toxocara canis.* (veterinarians and physicians). Answers between 90 and 100% infestation rate were acceptable in this survey. This important information could in general not expected to be known by physicians, but was clearly communicated in the education campaign. Therefore a possible difference in knowledge due to the campaign could be measured in this way.
* *The estimated risk of Toxocara infection from direct contact between children and young dogs and cats* (veterinarians and physicians). Despite the fact that *Toxocara* infestation in young dogs and cats often exists, direct contact does not create high risk for zoonotic infection, because *Toxocara* eggs need maturation in the environment. Answers between 0 to 10% were acceptable.

Pet owners and non-pet owners were asked concerning knowledge if worms of animals are in general the same as in man, which is not the case. Besides of that, it was questioned if worms of animals could be infective for man (zoonotic risks). The attitude of deworming their animals, eventual influenced by the campaign, was compared by asking for the last actual anthelmintic treatment. Finally recognition of the names roundworm and *Toxocara* was investigated by showing a preprinted list with worm names.

Statistical methods

Statistical analysis was performed using the 'Yates corrected Chi square' in Statistix 3.5 (NH Analytical Software, St. Paul MN) applying a significance level of 5%.

Results

Veterinarians

The response rate in the *first survey* was 95% (n = 200). Eighty three per cent were male; the actual number was 80% (14). The veterinarians were graduated 13.7 years on average and worked in large animal practice (6%), mixed practice (55.5%) or companion animal practice (38.5%). Approximately one third was working as single practitioner. Fourty per cent were practicing in a city and 60% in the country or small villages.

The results of the answers from veterinarians are listed in Table 1. Regarding the interval of deworming during the first 12 weeks of age, 5 participants never advised to repeat the first anthelmintic treatment in puppies and 18 do not recomment this in kittens. Two respondents never advised routine deworming of adult animals. No difference in knowledge of the several topics could be established between the kind of veterinarian (sex, year post graduation) nor practice (location, companion or large animal, solitary or associated). Finally, 79% of the veterinarians wished more general information to the public on deworming of animals. It was considered important that information should be given through veterinarians without causing panic or an impression of advertisement.

topic	survey 1	survey 2	p-value ($\chi^2_{df=1}$)
age first deworming			
pup average (days)	28 (sd 14.4)	26 (sd 14.1)	
correct	29% (59/200)	33% (35/105)	0.58
kitten average	32 (sd 16.4)	31 (sd 14.5)	
correct	51% (100/196)	52% (54/103)	0.91
interval deworming			
pup average (days)	30 (sd 14.6)	26 (sd 13.9)	
correct interval	14% (21/147)	37% (28/76)	0.0002
kitten average	31 (sd 15.7)	28 (sd 14.8)	
			0.0033

correct interval	14% (18/128)	33% (23/70)	
deworming adults advice routine deworming ante partum post partum	 76% (151/199) 90% (134/148) 17% (25/146)	 93% (97/104) 92% (90/98) 32% (31/98)	 0.0003 0.90 0.013
zoonotic risks *T. canis* + *T. cati* correct *T. canis* alone *T. cati* alone	 73% (146/200) 9% (17/200) 2% (3/200)	 90% (95/105) 8% (8/105) 1% (1/105)	 0.0006
infection rate pups average rate correct rate	 84% (sd 19.6) 61% (107/177)	 81% (sd 22.1) 50% (52/97)	 0.33
infection risk children average risk correct risk	 30% (sd 29.4) 47% (72/154)	 39% (sd 31.6) 29% (26/90)	 0.009

Table 1: Results of surveys among veterinarians before and after the education campaign

The response percentage of the *second survey* was 94% (n = 105). Seventy per cent were male (actual number: 78%). The results are listed in Table 1. Two and 8% of the veterinarians still do not advise to repeat deworming during the first 12 weeks of age for puppies and kittens respectivily or did not know an answer. No improvement could be established in the advised age of first deworming for puppies and kittens, ante partum deworming, knowledge of the infection rate of puppies and infection risk of children. Finally, 104 veterinarians (99%) said to have noticed the education campaign by the government.

Physicians

The response percentage in the *first survey* was 64% (n = 135). Eighty one per cent were male; the actual number was 81% (18). The participants were graduated 16.3 years on average. Two per cent were temporarily employed; 59% working as single practitioner (actual percentage 52%) and the remaining group were in partnerships (2 to 6 practitioners per clinic). Sixty one per cent were practicing in a city. The results of physicians are presented in Table 2.

Topic	survey 1	survey 2	p-value ($\chi^2_{df=1}$)
Zoonotic risks			
T. canis + *T. cati* correct	44% (60/135)	44% (46/105)	1.00
T. canis alone	17% (23/135)	8% (8/105)	
T. cati alone	3% (4/135)	1% (1/105)	
infection rate pups			
average rate	51% (sd 32.4)	57% (sd 32.6)	0.95
correct rate	13% (17/69)	15% (16/60)	
infection risk children			
average risk	25% (sd 27.1)	39% (sd 31.6)	0.12
correct risk	55% (52/94)	42% (32/76)	

Table 2: Results of surveys among physicians before and after the educational campaign

No difference in knowledge could be established between the kind of physician (sex, year post graduation, university of graduation) nor practice (location, solitary or associated). The question about the need of more general information to the public about zoonotic risks of *Toxocara* was agreed by 70% of the physicians; 23% disagreed and 7% did not answered.
The response percentage of the *second survey* among physicians was 70% (n = 105). Eighty five per cent were male. The regional representation of the respondents resembled in both surveys the actual regional distribution in the Netherlands. No difference of knowledge at all was found between the two surveys. Nearly half of the respondents (47%) had heard about the education campaign in 1993, but 7 of them remarked 'vague' or 'seen but not read'. Therefore it can be stated that maximal 42 participants (40%) noticed the campaign.

Pet owners and non-pet owners

In the *first survey* the average age of the 263 dogs and 248 cats in the households amounted 6.6 years. The answers of the pet owners and non-pet owners from both surveys are showed in Table 3. Thirty per cent of the respondents did not give an answer to the question if worms of animals were identical to the worms of man. To the question which worms could be recognised from a preprinted list, 6% of the participants did not know any name at all.
In the *second survey* 291 dogs and 229 cats with an average age of 6.7 years were present in the households. From the respondents a similar percentage respondents as in the first survey

recognized the zoonotic risks of worminfections of pets, despite the fact that 20% of this group said they had been informed about roundworms in the past year. Except a significant decrease of the opinion that worms of animals are identical to worms of man, no difference in knowledge or deworming could be established between the two surveys.

In total, 8.2% of all participants reported to be informed by the education campaign about roundworms in 1993: 60 (11.6%) dog and cat owners and 4 (1.5%) non-pet owners. The sources of information were in 53% of the cases the veterinary surgeon and 41% the brochure. The remaining 6% was not explained. Information from radio, television and weekly newspapers was not remembered by the participants. From the 60 pet owners who were informed by the campaign, 15% had dewormed their animals as a consequence of this information. In 1993, 12.5% of the pet owners had been advised to deworm their animals. This advice was mainly given by the veterinarian (80%). Other sources were the breeder (9%), family or friends (3%) and the physician (3%). The remaining 5% was not explained.

topic	**survey 1**	**survey 2**	**p-value ($\chi^2_{df=1}$)**
worms animals identical	33% (201/611)	22% (172/781)	0.0007
zoonotic risks	46% (281/611)	47% (367/781)	0.79
actual last deworming:			
0 - 6 months ago	45% (227/511)	40% (181/456)	0.16
6 - 12 months ago	17% (87/511)	15% (66/456)	0.32
> 1 year ago	29% (148/511)	33% (152/456)	0.16
never	10% (49/511)	13% (57/456)	0.18
recognised name:			
roundworm	73% (373/511)	78% (405/520)	0.08
Toxocara	7% (36/511)	8% (42/520)	0.61

Table 3: Results of surveys among pet owners & non-pet owners before and after the education campaign

Discussion

Veterinarians

Despite of the fact that the government campaign was well recognized by veterinarians and a measurable improvement in knowledge could be established for several topics, the overall

basic knowledge of a common zoonosis like Toxocarosis is still inadequate. Besides of the zoonotic risks, all other questions were correct answered by half or less of the respondents. It should be considered that the power of this survey was 80% to detect a minimal difference of 20% with a significance of <5%. Less than close to 100% correct answers is not acceptable for veterinarians. Similar results were obtained after surveys in the USA by Kornblatt and Schantz (11) who concluded that ‘current veterinary practices concerning the zoonotic aspect of *T. canis* infections are inadequate' and Harvey, Roberts and Schantz (10) who drawed the same conclusion: 'we conclude that current veterinary practices are inadequate for maximal prevention of environmental contamination with eggs of these intestinal helminths'.

The results from this survey in the Netherlands suggest that the way of information, as performed in the education campaign by the government, had a useful effect, but veterinary continuing education of parasitology (with special reference to zoonoses) is still needed.

Physicians

From the results of this study, for Dutch physicians it can be assumed that human toxocarosis is a nearly unknown phenomenon. Despite all efforts by the government to provide information concerning epidemiology and zoonotic aspects of *Toxocara*, the impact of the campaign was very low and no difference could be established in any discussed topic. It cannot be expected that physicians inform their patients sufficiently about the risks of pica and hygiene during and after playing in sandpits, parks, gardens and other high risk sources of (infective) *Toxocara* eggs. Education during the medical training of physicians and continuing education is recommended as well as having a critical look on the way of communication of important (new) medical information by the government. This should have a higher impact than the ways described in this article.

Pet owners and non-pet owners

The knowledge of the Dutch public about zoonotic risks of dog and cat roundworms and their attitude concerning deworming was as largely absent in both surveys and was not influenced by the education campaign. Direct education of (non-) pet owners about animal and public health in a campaign as performed in the Netherlands by the government has proven to be not effective. Unless all efforts and costs to communicate a simple message about the importance of deworming pets for at least public health reasons, a very low impact could be established. Such information to the public will probably be better communicated by pet care related professions as demonstrated for veterinarians in this survey. Employees of pet shops and

shelters as well as breeders can be very suitable for this purpose. These groups understand in general the need of education and are interested in getting important and sufficient information to use in contacts with their clients. In the human field careful information about this subject should be given by physicians, infant welfare and school doctors.

Finally it can be very helpful if at registration of veterinary anthelmintics, the producers or distributors of these drugs are stimulated to communicate complete and comparable information on labels and leaflets. The difference between larvicide and adulticide anthelmintics and the different deworming schedules in young animals should be stressed.

It can be concluded that the knowledge of *Toxocara* and toxocarosis is low or nearly absent in all investigated groups in our study before and after the education campaign. Continuation of education is therefore recommended. The ways of communication should be chosen carefully to obtain an optimal reach of the message.

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Chapter 9

Summarizing discussion

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Introduction

The purpose of this thesis is to study some aspects of the epidemiology of ascarids of dogs and cats, in particular *Toxocara canis* and *Toxocara cati*, in the Netherlands. This knowledge is essential to obtain a better understanding of the dynamics of this zoonotic infection which can then be used in effective prevention and education programmes. *Toxocara* infection is the (covert) infection following ingestion of *Toxocara* eggs, or ingestion of larvae that can lead to (overt) clinical disease, which is called toxocarosis.

In Chapter 1 and Chapter 2, a review of the literature is given regarding toxocarosis in dogs and cats and in man respectively. The life cycles of *Toxocara canis* and *T. cati* were described by Sprent in the late fifties for the first time (34, 35). He demonstrated that larvae of both *Toxocara* species migrated in the body of hosts as well as in paratenic hosts and may cause damage in organs, muscles and nervous tissue before they encapsulate and become dormant larvae. A few years earlier, in 1952, it became obvious that man could act as host since a nematode larva was isolated in a liver biopsy from a 2.5 year old child and identified as *Toxocara* spp (4).

A recent survey among 800 healthy individuals in the Netherlands (1995) revealed that between 10% of children up to 10 years of age and 30% of adults had antibodies against *Toxocara*. This reflects an infection with the larvae of this parasite (30). *Toxocara* larvae have the ability to survive in the tissues for at least 10 years (16). The role and importance of both *T. cati* and *T. canis* as causative parasites for infection is not well known. To obtain a better understanding, differentiation of *Toxocara* antibodies in sera of infected human is required.

Most infections are mild and resemble the symptoms of influenza. A minority of patients have clinical manifestations after massive invasion of larvae, called visceral larva migrans syndrome (VLM) (15, 17). In rare cases, migrating *Toxocara* larvae can induce an ocular larva migrans syndrome (OLM) (37). Finally, a third clinical syndrome is described and called covert toxocarosis (39). A relationship between *Toxocara* seroprevalence, the prevalence of asthma, elevation of serum IgE concentration, the presence of allergen-specific IgE and eosinophilia was described in 1993. It was concluded that in asthmatic children infection with *Toxocara* might boost allergic manifestations (9).

Prevention of the infection is based on measures such as appropriate health care for pets, including regular anthelmintic treatments, preventing pollution of the environment with faeces and promoting responsible pet ownership (38). Furthermore, precautions based on hygiene are

required. To achieve such preventive measurements, sufficient information by education is necessary (18).

This thesis is divided into three parts. Part I consists of a review of the literature as a general introduction. In Part II the results of surveys in the Netherlands are reported which determine the prevalence of *Toxocara* infections and their eventual risk factors. Part III presents the effect of preventive measurements such as deworming and the impact of education on awareness of *Toxocara*.

Prevalence of *Toxocara* and risk factors for infection

A survey on the prevalence of intestinal ascarids in the Netherlands was published in 1980 and was based on examination of a selected group of dogs during the period 1972-1977 (33). A survey in Utrecht in 1993 on soil contamination revealed contamination with *Toxocara* eggs in public parks (mainly *T. canis*) and in sand-boxes (mainly *T. cati*) (24). These findings confirmed the presence of infected dogs and cats and gave an indication of the infection pressure but did not elucidate the number and origin of the infected animals.

For this reason the actual prevalence of patent *Toxocara* infections in Dutch dogs and cats was investigated in several surveys. The possible presence of risk factors in relation to breeding, deworming and zootechnical aspects (e.g. feeding, housing, pet care, cleaning and desinfecting) was also investigated.

In Chapter 3, the results of surveys on dogs and cats from Dutch households in two different urban areas and a survey on stray cats are presented to determine the prevalence of intestinal nematodes, based on faecal examination. The prevalence found in the privately owned dogs and cats was 2.9% for *T. canis* and 4.7% for *T. cati* respectively. The percentage of positive samples was significantly higher in young animals than in older animals. In 21% of the investigated stray cat faecal samples, *T. cati* eggs were identified. Based on some reports in the literature (27, 40, 14), the sensitivity of the faecal floatation method for *Toxocara* eggs was estimated as 51%. The actual infection rate in this survey may therefore be double the apparent percentage. In household animals this is therefore not exceeding the 10% infection rate. Stray cats, however, may have an infection level in exces of 40%. From these results some conclusions can be formulated. Even after correction for the sensitivity of the test used, the prevalence of *Toxocara* infections in dogs and cats in households is not as high as was suggested for the Netherlands (a prevalence up to 39% for *T. canis* in dogs and 60% for *T.*

cati in cats) extrapolated from a Belgian situation (42) and as reported in 1980 (33). The results agree, however, with more representative reports from Germany (19, 20) and from the U.S.A. (25). The existence of infected pets should make the veterinary practitioner and pet-owner aware of the continuing need for regular deworming, with emphasis on young animals. Even a relatively low percentage of 5 to 10% infected animals should never be neglected, because even that figure represents more than 200.000 Dutch dogs and cats that daily shed billions of *Toxocara* eggs in the environment. Stray cats with a considerably higher infection rate are responsible for another aspect of environmental contamination. The Dutch Society for Animal Welfare estimate hundred thousands to a million of stray cats to be present in the Netherlands. A reliable deworming programme for this group of animals is needed, but will for practical reasons be very difficult to perform.

In Chapter 4 and Chapter 5, the results of respective surveys in dog and cat breeding colonies are presented. Dogs were found to have patent *Toxocara* infections in one third of the investigated kennels and the average prevalence of the investigated adult dogs and puppies was 8% and 15% respectively. The number of litters per year and regular import of new dogs were found to be risk factors for *Toxocara* infection. Moreover *T. canis* eggs were demonstrated in 20% of house and kennel dust samples and in as much as 50% of soil samples.

In contrast to these findings, only 1% of the adult cats in breeding colonies were shedding *T. cati* eggs. These animals originated from 8% of the investigated catteries. Dust and soil samples were free from *Toxocara* eggs.

Compared to the household dogs and cats, the prevalence of patent *Toxocara* infections can be considered as high in dog breeder kennels and relatively low in catteries. The contamination of the soil in dog kennels with *Toxocara* eggs was remarkably higher than in Dutch public parks (24). For this reason, the infection pressure for canine intestinal parasites can be considered as higher than, for example, individual household dogs. Two-thirds of bitches were dewormed after parturition and only one-third of breeders dewormed their dogs conforming to a recommended deworming scheme (6). In the literature, some reports can be found concerning prevalence in different types of kennels (12, 21, 22, 32). The *T. canis* prevalence in these studies varied between 5% and 15% which coincides closely the findings for the Netherlands in this report (8% - 15%)

To reduce the risks for zoonotic infection, more information about deworming strategies and the use of effective anthelmintics should be presented to dog breeders. They should also be educated about their responsibility regarding the prevention of toxocarosis. The veterinarian

can play an important role in this and he or she should spend sufficient time informing puppy owners.

Cats from catteries do not seem to be a great risk for *T. cati* infection in man, despite the fact that only half of the breeders dewormed their queens after parturition and not more than 12% of the breeders followed the advised deworming schedule. Moreover half of the breeders administered the first anthelmintic treatment for kittens at ages over eight weeks when first patent infections can already be established.

It can be suggested therefore that transmammary *Toxocara* infection seems to be low in breeding cats, which can be caused by limited access to environment and wildlife. The irregular deworming programmes may also have some influence on this. Nevertheless, deworming schemes can be optimised and more education and information on these topics is advised.

In Chapter 6 the hypothesis was investigated that if, as result of oestrus somatic, larvae in the tissues are activated it may permit these or larvae from a newly acquired infection, to undergo tracheal migration in adult bitches with subsequent intestinal maturation. As result patent *T. canis* infection was more often seen in bitches during metoestrus (11). If this finding could be confirmed, oestrus must be considered as a risk factor for *T. canis* infection and adaptation of the currently advised deworming schemes may be required. Prolactin was suggested as the triggering factor (11).

A group of cyclic beagles was monitored over a two year period by determination of white blood cell count, eosinophil count, *Toxocara* titre and prolactin concentration in plasma and by performing faecal examination during the post-oestrus period up to 140 days. During 23 Oestrous cycles of 15 dogs, two young animals were found to be shedding *Toxocara* eggs at 60 and 140 days after onset of the luteal phase respectively. These time points deviate considerably from the earlier described metoestrus (11). Some observed moderate alterations of plasma *Toxocara* titres in cyclic dogs which may be an indication of the existence of stimulus by (reactivated?) *T. canis* larvae, but differences in concomitant eosinophil counts did not occur. Taking into account the faecal examination results, it was concluded that it is not likely that oestrus is not a higher risk for patent *T. canis* infection. Finally, an elevation of prolactin levels during the luteal phase was not observed in contrast to the pregnant control dogs and therefore not considered as having influence. It can be questioned if these results significantly differ from animals under other circumstances such as in an environment heavily infected with *T. canis* eggs or from dogs belonging to more varied groups of breeds or age. Another group that certainly deviates is pseudopregnant bitches which show an elevation of

prolactin concentration (31). Further research is therefore needed in these animals to get a better understanding of whether or not patent *T. canis* infections will develop.

Anthelmintic treatment as a preventive measure

Anthelmintic strategies in dogs and cats are an important part of the preventive measures to expel worms from the intestinal lumen and to stop the *Toxocara* egg output. Deworming schemes, adapted to the life cycle of the parasite, have been developed and advised (2, 6). Benzimidazoles are in general highly effective against gastro-intestinal nematodes of dogs and cats (3, 8, 23, 29, 41), have significant larvicidal effects (1, 23) and are virtually without toxicity at the therapeutic dose rates (7, 13). Oxibendazole (OBZ) is such a benzimidazole compound and indicated for several nematodes of dogs and cats (5, 10). Efficacy after single administration in these animals was not investigated or reported. Trials were therefore performed to investigate the anthelmintic efficacy and safety of OBZ in naturally acquired nematode infections in dogs and cats and in litters of puppies under field conditions.

In Chapter 7 the results of these experiments are presented. A single dose of 15 mg oxibendazole/kg body weight reduced faecal worm egg counts by 97.6% for *T. canis* in dogs and 96.7% for *T. cati* in cats. In a second trial, litters of puppies were treated twice on two consecutive days with the same dosage at 2, 4 and 6 weeks of age. During the investigation period between 3 and 7 weeks of age, 95% of the puppies did not shed *T. canis* eggs. This percentage was significantly higher compared to the 85% result after a single treatment. Side effects were rare and only recorded in young animals. Based on the guidelines for evaluating the efficacy of anthelmintics for dogs and cats issued by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.), it was concluded that OBZ is an effective and safe anthelmintic against *Toxocara* when used in accordance with the recommended dosage and deworming schedule. For puppies, a two day treatment with OBZ can be advised. With this information a better understanding of the use of this anthelmintic is achieved under circumstances resembling field usage and it will now be possible to improve the efficacy by adapting the treatment schedule for puppies. Further experiments should be carried out with a three day treatment in puppies with oxibendazole every two weeks to evaluate if 100% efficacy under field conditions can be achieved.

Impact of information and education

When a reliable impression about the *Toxocara* prevalence in different groups of dogs and cats in the Netherlands is obtained, some risk factors are determined or excluded and the efficacy of an anthelmintic product under field conditions investigated, better information can be provided to the public, veterinarians and physicians. Education about public health risks of *Toxocara* and the social concepts of responsible pet ownership is considered necessary (36).

In 1993, the Dutch Ministry of Public Health, Welfare and Sport, Department of Veterinary Inspection, started such a campaign about *Toxocara* and toxocarosis to inform the Dutch population. Previously, veterinarians and physicians were informed about this project and updated with knowledge of *Toxocara* epidemiology and toxocarosis to be prepared to answer questions from their clients.

Little or no information was available at this time about the actual knowledge of these professions and the general public. Chapter 8 describes the results of interviews of vets, physicians and the public before and after the education campaign. With the information collected, the knowledge and attitudes could be investigated and the effect of the education campaign evaluated.

The campaign was well recognised by veterinarians and a significant improvement in knowledge was established for several topics. However, the overall basic knowledge was still considered to be inadequate. Beside the zoonotic risks, all other questions were correctly answered by only 50% or less of the respondents. Similar results were reported from the USA (18, 26). For Dutch physicians, human toxocarosis seemed to be a fairly uncommon disease which may be neglected. Despite the efforts by the government, the impact of the campaign was very low and no difference could be established in any topic. Finally, the knowledge of the Dutch public about the zoonotic risks of dog and cat ascarids and deworming was largely absent in both surveys and was not improved by the education campaign.

It was concluded that the knowledge of *Toxocara* and toxocarosis is moderate among veterinary practitioners but low or nearly absent in the other investigated groups in this study before and after the education campaign. Continuation of education is therefore recommended, but other ways of communication are required to obtain a better reach of the message.

With the knowledge from the results of the studies in this thesis and the information from the recent pilot study in the Netherlands (30), a general conclusion can be formulated that *Toxocara* and toxocarosis certainly play a role in the Netherlands. An average seroprevalence of 19% for man agrees with ± 3 million infected Dutch inhabitants. Even if only 1% of this

group showed clinical disease, this would amount to 30.000 patients per year. The actual number of cases is therefore probably much higher than expected by physisicians, because toxocarosis has been proven to be an unknown disease and there is no national mandatory reporting. Beside the emotional impact for patients which cannot be financially expressed, the mean cost of an adult toxocarosis patient caused by medical expenses and loss of days of labour was estimated in 1989 as £ 620 (28).

Continuing information and education is therefore required and efforts should be made to achieve more awareness in the professions that are involved in human and veterinary medicine as well as with dogs and cats.

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Chapter 10

Samenvatting

Dit proefschrift beschrijft de onderzoeken naar een aantal aspecten van de epidemiologie van *Toxocara canis* en *Toxocara cati*, spoelwormen van de hond en de kat, in Nederland. Met de hierdoor verkregen kennis kan het verloop van deze zoönose beter worden begrepen en gerichtere voorlichting worden gegeven over het voorkomen van de infectie.

In Hoofdstuk 1 en Hoofdstuk 2 wordt een overzicht gegeven van de literatuur over *Toxocara*-infecties bij hond en kat respectievelijk de mens. Al vanaf de vijftiger jaren is de cyclus van deze spoelwormen volledig bekend inclusief de besmetting bij de mens, die als gastheer van de larvale stadia van *Toxocara* kan optreden. Een in 1995 door het Rijksinstituut voor Volksgezondheid en Milieuhygiëne (RIVM Bilthoven) uitgevoerde pilot-study gaf te zien dat gemiddeld 19% van de 800 onderzochte (gezonde) Nederlanders antistoffen had tegen *Toxocara*. Dit betekent dat een *Toxocara*-infectie aanwezig was of had plaatsgevonden. De larven van deze parasiet kunnen wel 10 jaar overleven voordat ze afsterven en worden opgeruimd door het lichaam. De meeste infecties manifesteren zich als voorbijgaande griepachtige verschijnselen. In een klein aantal gevallen kunnen klinische symptomen optreden als gevolg van de afweerreactie van het lichaam op een grote hoeveelheid rondtrekkende larven. Dit wordt viscerale larva migrans syndroom (VLM) genoemd. In zeldzame gevallen kan een larve in het oog terechtkomen en daar een ontsteking veroorzaken die vaak resulteert in blindheid. Deze vorm wordt oculaire larva migrans syndroom (OLM) genoemd. Tenslotte is er een derde toxocarosis-syndroom beschreven (covert toxocarosis). Enkele jaren geleden werd in een ander Nederlands onderzoek een relatie vastgesteld tussen *Toxocara*-seroprevalentie en de prevalentie van astma, een toename in de serum IgE-concentratie, het voorkomen van allergeen-specifiek IgE en een toename in het aantal eosinofielen. Geconcludeerd werd dat er een tendens was dat allergische fenomenen bij kinderen met een aanleg voor astma, wordt versterkt door een *Toxocara*-infectie. Vooral dit laatste geeft het belang aan van een optimale preventie die is gebaseerd op drie groepen van maatregelen: voorkomen van besmetting van het milieu met *Toxocara*-eieren, het betrachten van hygiëne, vooral door kinderen, en het geven van voorlichting.

Dit proefschrift bestaat globaal uit drie onderdelen. Na een literatuuroverzicht als algemene introductie worden de resultaten worden besproken van onderzoeken naar de prevalentie van *Toxocara* in Nederland bij verschillende groepen honden en katten inclusief de eventuele aanwezigheid van risicofactoren. Tenslotte worden de resultaten van onderzoek naar het effect van preventieve maatregelen zoals ontwormen en het verstrekken van voorlichting over deze onderwerpen besproken.

In Hoofdstuk 3 worden de resultaten van veldonderzoeken naar de prevalentie van *Toxocara* en andere maag-darmnematoden besproken. Allereerst zijn in twee regio's in Nederland, bestaande uit een stad met voorsteden en omringende dorpen, eigenaren van honden en katten ondervraagd en ontlastingsmonsters van aanwezige huisdieren verzamelend. Verder zijn in enkele grote steden fecesmonsters van zwerfkatten verzameld. Het besmettingspercentage bleek te liggen op 2.9% voor *T. canis* bij de hond en 4.7% voor *T. cati* bij de kat. Jonge dieren bleken significant vaker besmet te zijn dan volwassen dieren. Bij de zwerfkatten werd in 21% van de onderzochte fecesmonsters *T. cati* eieren gevonden. Deze cijfers zijn echter veel lager dan hetgeen regelmatig wordt gesuggereerd op basis van onderzoeken uit België en komen meer overeen met de situatie zoals beschreven in Duitsland. De aanwezigheid van huisdieren die een *Toxocara*-besmetting hebben en eieren met de ontlasting uitscheiden geeft aan dat het noodzakelijk blijft om honden en katten regelmatig te ontwormen en dat de dierenarts en eigenaar hier blijvend aandacht aan moeten besteden. Zwerfkatten blijken verantwoordelijk te zijn voor een deel van de omgevingsbesmetting met *Toxocara*-eieren. Hoewel deze dieren daarom eigenlijk regelmatig ontwormd moeten worden zal het, gezien de omstandigheden waaronder ze leven, zeer moeilijk zijn hiervoor een betrouwbaar programma te ontwikkelen.

In Hoofdstuk 4 en Hoofdstuk 5 worden soortgelijke onderzoeken bij honden- en kattenfokkers besproken. Het bleek dat in een derde van de onderzochte kennels honden met een *Toxocara*-besmetting aanwezig waren. Het gemiddelde besmettingspercentage van volwassen honden en puppies bedroeg in alle onderzochte kennels respectievelijk 8% en 15%. Een toenemend aantal nesten per jaar en het regelmatig aankopen van nieuwe honden bleek de kans te vergroten op een *Toxocara*-infectie. In eenvijfde van de stofmonsters uit het woonhuis en kennels van de hondenfokkers en in de helft van de grondmonsters van uitlaat- en speelweiden werden geëmbryoneerde *T. canis*-eieren gevonden. Van alle onderzochte volwassen katten in catteries bleek gemiddeld 2% besmet te zijn met *T. cati*. Deze dieren waren afkomstig uit 8% van de onderzochte catteries. De ontlasting van de onderzochte kittens was in alle gevallen negatief. In de onderzochte stof- en grondmonsters werden geen *Toxocara*-eieren aangetroffen.

In vergelijking met de besmettingsgraad van honden en katten in Nederlandse huishoudens kan gesteld worden dat de prevalentie van patente *Toxocara* infecties hoog is bij honden in de onderzochte kennels en laag in de catteries. De grondmonsters van de hondenkennels waren veel vaker besmet dan de onderzochte Nederlandse parken. De infectiedruk ligt daarom hoger in kennels dan bij individueel gehouden honden. Daarnaast werden te weinig teven na de geboorte ontwormd, terwijl slechts eenderde van de hondenfokkers de honden ontwormde

volgens het geadviseerde ontwormingsschema. Om het risico van besmetting van de honden maar ook de mens te verkleinen, is het nodig dat meer informatie wordt verstrekt aan hondenfokkers over ontwormen en het gebruik van effectieve ontwormingsmiddelen. Daarin kan de dierenarts een belangrijke rol spelen door hieraan voldoende aandacht te besteden en door voorlichting te geven aan nieuwe eigenaren van puppies.

De fokkatten in catteries lijken nauwelijks risico te lopen op een *T. cati* infectie, ondanks het feit dat slechts de helft van de fokkers de poezen na de partus ontwormen en niet meer dan 12% van de fokkers het geadviseerde ontwormingsschema volgt. Bovendien geeft iets meer dan de helft van de kattenfokkers kittens de eerste ontworming op een leeftijd van 8 weken of ouder, een leeftijd waarop al volwassen, ei-producerende wormen aanwezig kunnen zijn. De lactogene besmetting lijkt bij fokkatten daarom klein te zijn. Dit is misschien het gevolg van een kleinere blootstelling aan besmette grond en aan mogelijke prooidieren buitenshuis. Zelfs het ontwormen, hoewel dit niet altijd regelmatig en volgens schema geschiedt, heeft hierop nog invloed. Het ontwormingsschema kan echter in de meeste gevallen nog worden verbeterd en voorlichting hierover wordt daarom geadviseerd.

In Hoofdstuk 6 is de in de literatuur vermelde hypothese onderzocht of de oestrus bij teven een activering van somatische larven kan induceren gevolgd door een tracheale migratie, met als gevolg patente infecties gedurende de metoestrus. Indien dit het geval is, dan kan de loopsheid als risicofactor worden beschouwd en is aanpassing van het geadviseerde ontwormingsschema noodzakelijk. Een groep van 15 vrouwelijke beagles werd gedurende twee jaar vervolgd waarbij rond elke loopsheid, tot 140 dagen daarna, bloed- en ontlastingsmonsters werden verzameld. Het bloed werd onderzocht op het aantal totaal aantal wite bloedcellen, eosinofile bloedcellen, prolactine-concentratie en *Toxocara* titers. Tijdens 23 onderzochte oestrische cycli bleken twee jonge onderzochte honden *Toxocara* eieren uit te scheiden op 60 en 140 dagen na de aanvang van de luteale fase. Deze tijdstippen wijken af van de beschreven metoestrus van 30 tot 60 dagen. In het bloed van sommige dieren konden veranderingen van de *Toxocara* titer worden waargenomen. Dit wijst erop dat activiteit van *Toxocara* larven in het lichaam aanwezig is. Er werden echter geen fluctuaties van eosinofiele bloedcellen waargenomen. In combinatie met de resultaten van het ontlastingsonderzoek is geconcludeerd dat de oestrus geen groter risico vormt voor het verkrijgen van een patente *Toxocara* infectie.

Het ontwormen van honden en katten vormt een belangrijk onderdeel van de preventieve maatregelen om wormen uit het maag-darmkanaal af te drijven en de uitscheiding van

Toxocara-eieren te voorkomen. Hiervoor zijn ontwormingsschema's geadviseerd die zijn ontwikkeld op basis van de cyclus van de parasiet. Benzimidazolverbindingen vertonen over het algemeen een goede werkzaamheid tegen maag-darmwormen bij honden en katten inclusief de onvolwassen stadia en zijn weinig toxisch voor de gastheer in de voorgeschreven dosering. Oxibendazol (OBZ) is een dergelijke benzimidazolverbinding die is geregistreerd voor hond en kat, maar waarvan de werkzaamheid tegen maag-darmwormen na eenmalige dosering van 15 mg/kg lichaamsgewicht nooit is gepubliceerd. Daarom is dit onderzocht bij natuurlijk geïnfecteerde honden, katten en puppies onder praktijkomstandigheden.
In Hoofdstuk 7 worden hiervan de resultaten weergegeven. Een enkelvoudige behandeling met OBZ in de geadviseerde dosering van 15 mg/kg lichaamsgewicht reduceerde het aantal wormeieren in de ontlasting met gemiddeld 97.6% voor *T. canis* bij de hond en 96.7% voor *T. cati* bij de kat. In een tweede onderzoek werden bij fokkers puppies op twee opeenvolgende dagen ontwormd met dezelfde dosering op een leeftijd van 2, 4 en 6 weken. Het resultaat was dat bij gemiddeld 95% van de puppies gedurende de gehele periode geen *T. canis* eieren werden gevonden in de feces. Dit percentage was significant hoger dan de 85% negatieve monsters die werden verkregen na een eendaagse dosering op een leeftijd van 2, 4 en 6 weken. Bijwerkingen werden weinig gemeld en alleen bij jonge dieren. Op basis van de richtlijnen van de World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) voor de werkzaamheid van anthelmintica voor honden en katten werd geconcludeerd dat OBZ een effectief en veilig anthelminticum is voor preventie van *Toxocara* bij toepassing in de voorgeschreven dosering en volgens het geadviseerde ontwormingsschema. Voor puppies wordt een tweedaagse behandeling geadviseerd. Met deze informatie kan een betere indruk worden verkregen over het gebruik van dit ontwormingsmiddel onder praktijkomstandigheden en is het mogelijk om het ontwormingsschema voor puppies te optimaliseren.

Nu meer inzicht is verkregen in de besmettingsgraad van *Toxocara* bij honden en katten in Nederland, enkele risicofactoren zijn vastgesteld c.q. uitgesloten en de werkzaamheid van een ontwormingsmiddel onder praktijkomstandigheden is geëvalueerd, kan beter onderbouwde informatie worden verstrekt.
In 1993 werd onder verantwoordelijkheid van het Staatstoezicht op de Volksgezondheid een voorlichtingscampagne gestart over *Toxocara* en toxocarose, gericht op de Nederlandse bevolking. Voorafgaande aan de campagne werden alle dierenartsen en huisartsen in kennis

gesteld van de campagne om ze voor te bereiden op eventuele vragen. Het was niet bekend of er op dat moment voldoende kennis over deze onderwerpen aanwezig was.

Hoofdstuk 8 beschrijft de resultaten die zijn verkregen uit telefonische enquêtes van huisartsen en dierenartsen en uit enquêtes die werden afgenomen tijdens het bezoeken van Nederlandse huishoudens voor- en na de voorlichtingscampagne. Hiermee kon de kennis op het gebied van *Toxocara* en toxocarose worden onderzocht en het resultaat van de voorlichtingscampagne worden beoordeeld. De dierenartsen hadden nagenoeg allen vernomen van de campagne en er kon op diverse onderdelen een verbetering in de kennis worden vastgesteld. In het algemeen was de kennis nog niet optimaal. Ten aanzien van de Nederlandse huisartsen werd geconcludeerd dat bekendheid over deze belangrijke zoönose, ook na de informatie-voorziening, afwezig was en dat de wijze van voorlichting over dit onderwerp had gefaald. Bij de Nederlandse bevolking bleek de kennis over de besmettingsrisico's van spoelwormen van hond en kat voor de mens nagenoeg afwezig te zijn in beide onderzoeken. Dit gold ook voor het standpunt ten opzichte van het ontwormen van huisdieren.

Met de gegevens uit dit proefschrift, samen met de informatie uit de pilot-study in 1995 van het RIVM, wordt geconcludeerd dat *Toxocara* en toxocarose ook in Nederland een belangrijke rol speelt. Het blijft daarom noodzakelijk om voorlichting te geven aan de bevolking en beroepsgroepen uit de medische en veterinaire sector en direct betrokkenen bij het fokken, houden en verzorgen van honden en katten. Om hiervan betere resultaten te verkrijgen dient de wijze van informatievoorziening kritisch bekeken te worden.