Toxoplasmosis in Belgian pet cats: recommendations for owners

Toxoplasmose bij Belgische huiskatten: aanbevelingen voor de eigenaars

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ABSTRACT

Pet cats live in close proximity with their owners and are considered a potential source of *Toxoplasma gondii* infection for humans. Four hundred and ten sera of healthy pet cats originating from different parts of Belgium were examined. The age of the cats varied from 3 months to 8 years. Nearly 27% of the cats tested positive for Toxoplasma IgG and/or IgM antibodies. Toxoplasma seroprevalence was lowest in Flanders (20.4%), followed by Brussels (30.9%) and Wallonia (35.0%). Seroprevalence increased with age: from merely 2% below 12 months of age, up to 47% at 7 years of age. Infection peaks were observed in the age group of 12-23 months (17.6% increase) and, remarkably, also in the age group of 6 years (14.0% increase).

The study suggests an overall chance of 5.5% for a seronegative cat to become infected within the next year. The risk of transmission of toxoplasmosis to the cat's owner is discussed.

SAMENVATTING

Huiskatten leven in de nabijheid van hun eigenaar en worden aanzien als een bron van toxoplasma-infectie bij de mens. Vierhonderd en tien sera afkomstig van gezonde huiskatten van 3 maanden tot 8 jaar oud, uit verschillende delen van België, werden onderzocht. Nagenoeg 27% van de katten was positief voor toxoplasma IgG- en/of IgMantilichamen. De toxoplasmaseroprevalentie was het laagst in Vlaanderen (20,4%), gevolgd door Brussel (30,9%) en Wallonië (35,0%). De seroprevalentie nam toe met de leeftijd van de kat, gaande van 2% bij katten jonger dan 12 maanden tot 47% bij katten van 7 jaar oud. Infectiepieken traden vooral op bij de leeftijd van 12 tot 23 maanden (17,6%) en bij katten van 6 jaar (14,0%).

De data geven aan dat een seronegatieve kat 5,5% kans heeft om in het volgende jaar een infectie op te lopen. Het risico op een transfer van toxoplasmose van de huiskat naar de eigenaar wordt besproken.

INTRODUCTION

Toxoplasma gondii is an obligate intracellular zoonotic parasite with high prevalence that infects almost all warm-blooded animals (Tenter et al., 2000). Only infection of the *felidea* can result in the shedding of millions of oocysts in the environment (Dubey, 1986). The sources of infection for carnivorous animals and humans are tissue cysts from infected meat and the ingestion of oocysts from a contaminated environment, while in herbivorous animals infection is restricted to the latter. The infection remains asymptomatic in most animals, except in sheep and goats, where infection during gestation leads to abortion and stillbirth. In Toxoplasma-seronegative pregnant women and immunocompromised patients, infection may lead to congenital and encephalitic toxoplasmosis, respectively (Montoya and Liesenfeld, 2004), and to ocular toxoplasmosis in healthy individuals (Gilbert et al., 2006). Education on toxoplasmosis has traditionally been directed to pregnant women (Breugelmans

et al., 2004), for whom consumption of infected meat is regarded as the main source of infection (Cook et al., 2000). However, the fact that strict vegetarians may seroconvert to T. gondii suggests that oocysts still play a role in the transmission of this parasite (Hall et al., 1999; Roghmann et al., 1999). Indeed, toxoplasmosis in humans has been linked to living in close proximity to Toxoplasma seropositive cats (Mac-Knight and Robinson, 1992; Pereira et al., 1992; Sukthana et al., 2003). Nowadays, pet cats leading an indoor life are mostly fed pre-packaged sterilized food and are considered a low risk for transmission of T. gondii to their owners. However, owners may infect their cat by offering raw meat as a treat, or occasional outdoor access may lead to infection via oocysts or predation of infected wildlife (Woods, 2003; Afonso et al., 2006).

The aim of this study was to evaluate the seroprevalence of *T. gondii* in Belgian pet cats; to determine whether this infection is equally distributed in the Belgian territory; and to evaluate the risk of transmission of this zoonotic parasite from cat to owner. We suggest guidelines for veterinary and medical doctors, to minimize the zoonotic risk that pet cats pose for patients susceptible to toxoplasmosis.

MATERIALS AND METHODS

Sera

Serum from healthy pet cats aged 3 months to 8 years was obtained from the National Reference Laboratory for Rabies at the Pasteur Institute of Brussels. These sera were collected between 2004 and 2007, complement-inactivated at 56°C for 30 minutes and conserved at -20°C. These sera were originally submitted for titration of rabies antibodies in accordance with the European pet travel scheme, to allow the owners to enter certain rabies-free countries with their pet cat. All sera were taken by private veterinary practitioners prior to the travelling of the animal outside the Belgian territory. All the animals were healthy at the time of sampling, as determined by general clinical examination. Per age group of one year, 31 to 50 samples were randomly selected. A minimum sample size of 31 animals/age group allowed sufficient power ($\beta =$ 0.80) to interpret differences as low as 3.6% between groups as statistically significant ($\alpha = 0.05, \sigma = 5\%$).

Indirect Immunofluorescence Assay

The presence of IgM and IgG antibodies against *T. gondii* in cat sera was evaluated by indirect immuno-fluorescence assay (IIFA). Fifty microliters of each serum diluted 1/50 in PBS was applied on slides coated with formalin-treated tachyzoites from the RH strain (Toxo-Spot IF, Bio-Mérieux, Marcy-l'Etoile, France) and put for 30 minutes in a 37°C incubator.

The slides were washed with PBS and incubated for 30 minutes at 37°C with 30 μ l of fluorescein isothiocyanate (FITC) conjugated goat-anti-cat IgM (μ)- or goat-anti-cat IgG (H+L)-FITC (KPL, Gaithersburg, MA) diluted 1/20 and 1/40 in PBS, respectively. After washing and drying, the slides were read with a fluorescence microscope (Carl Zeiss). The cut-off read-out of the fluorescence test was established at a dilution of 1/40 with both toxoplasma seronegative and sero-positive feline reference sera from our laboratory. The specificity and sensitivity of the Toxo-Spot IF test for IgG are 98.44% and 95.08%, respectively, while for IgM these are not detailed by the manufacturer.

Geographical data

The postal code of the cat owners was provided for all cat sera collected and the serum samples were grouped per Belgian province or federal region. Considering the highly uneven distribution of the samples in the different regions, the regional differences were not interpreted on a statistical basis and are merely informative.

Statistical analysis

Logistic regression was used to investigate the correlation between *Toxoplasma* seroprevalence and age, using SPSS 13 software (SPSS, Chicago, Illinois).

RESULTS

Seroprevalence to T. gondii in Belgian domestic cats

Of the 410 Toxoplasma IIFA tested cat sera, 26.8% was positive for IgG and/or IgM antibodies. On average, *T. gondii* specific IgM antibodies occurred in 3.3

Serological status of the pet cat	Suggested counseling guidelines
Seronegative or unknown	 Owners should be informed that there is a 5.5% chance that their seronegative cat will shed oocysts if exposed to <i>T. gondii</i> in the coming year, in other words, if the cat has outdoor access and is fed uncooked meat. This chance is considerably higher (17.6%) with cats aged 12-23 months. Cats must strictly be kept indoors and fed on sterile prepackaged food during the period that their owner is at risk, in order to prevent infection and transmission. Rodent control must be implemented if mice have been seen inside or in the vicinity of the house. Another family member should clean the litter box, and the feces should be removed daily. Contaminated surfaces should be cleaned with bleach. There is no need to remove the cat from the house.
Seropositive	 In cats younger than 6 years of age, a positive Toxoplasma antibody test ensures that the cat is not at risk for oocyst shedding and thus does not pose a direct risk for transmission of Toxoplasma to its owners. Cats aged 6 years or older may still represent a risk for oocyst shedding, irrespective of their immune status, in which case, the same measures as for seronegative cats must be implemented. There is no need to remove the cat from the house.

Table 1. Toxoplasma guidelines for veterinary practitioners, general practitioners and gynecologists when counseling cat owners at risk for toxoplasma infection.

cific IgM antibodies was observed in the age group of 7 years and this increase was continued in the age group of 8 years; $18.1 \pm 5.2\%$ (SEM). On average, 6.6 $\pm 2.4\%$ (SEM) of cats were IgM positive. Seroprevalence increased with age: from merely 2% below one year of age up to 47% in the age group of 7 years (Figure 1). Logistic regression on continuous age data in-

dicated that increase in age was positively correlated with increase in seropositivity (r=0.991, P < 0.0001). The increase in prevalence in successive age groups, calculated as the prevalence in age group (n) – the prevalence in age group (n-1), peaked in the age group of 12-23 months (17.6% increase) and remarkably also at 6 years of age (14.0% increase). In other age groups the increase of seroprevalence was merely 0 to 6.3%. The average increase in seroprevalence per life year was $5.5 \pm 2.1\%$ (SEM).

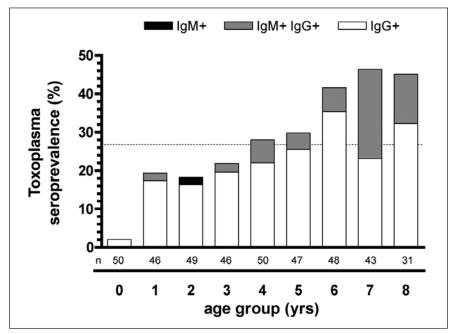


Figure 1. Toxoplasma seroprevalence per age group. The average toxoplasma seroprevalence is represented by a dotted line. (n) represents the number of cat sera tested per age group.

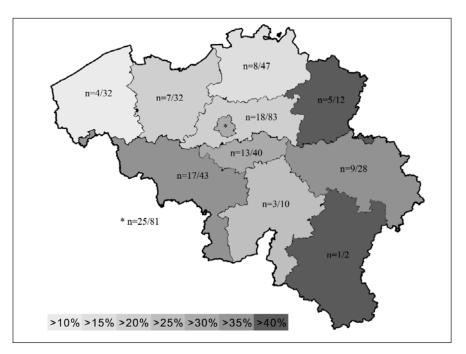


Figure 2. Geographical distribution of toxoplasma infection in pet cats. The data represent the prevalence per province, with n = number of seropositive cats/total number of tested cats.

Geographic distribution of *T. gondii* infection in cats

In order to evaluate whether Toxoplasma infection in Belgian pet cats was geographically evenly distributed, the results were grouped per province and region. Regional differences were observed and are shown in Figure 2. For the province of West-Vlaanderen the lowest seroprevalence was noted (12.5%), followed by Antwerpen (17%), Oost-Vlaanderen (21.9%), Vlaams Brabant (21.7%), Namur (30.0%), Brussel (30.9%), Liège (32.1%), Brabant Wallon (32.5%), Hainaut (39.5%) and Limburg (41.7%). Only 2 cat sera originating from the province of Luxembourg were collected, one of which one tested positive. The prevalence was 20.4% (n=42/206) in the Flemish Region, 30.9% in the Brussels Region (n=25/81) and 35.0% (n=43/123) in the Walloon Region.

DISCUSSION

In this study we show that in Belgian pet cats, the seroprevalence to T. gondii increases by 5.5% for every life year of the cat, with an average seroprevalence of nearly 27%. Overall, 6.6% of the cats tested positive for both IgM and IgG, indicating a recent infection before sampling (Lappin, 1996). Only one out of 410 tested cats (0.24%) showed IgM without IgG antibodies, which suggests that the infection took place 1-3 weeks earlier and that this cat might have been shedding oocysts at the time of sampling. IgM antibodies are elicited as early as 1 week post-experimental infection (pi) and may persist up to 3 months pi (Chavkin et al., 1994; Lappin, 1996), and IgG antibodies appear 2-4 weeks pi and may persist for life (Dubey et al., 1995; Lappin, 1996). In our study, the most important increase in seroprevalence was observed at the age of 12-23 months (i.e. 1-year-old cats) and there was also a moderate increase in 6-year-old cats. This reflects a primary infection in young cats and suggests that re-exposure to T. gondii may have occurred in 6-year-old cats. However, older cats have been shown to produce more IgM antibodies than younger cats (Campbell et al., 2004), and this age-related phenomenon may explain the high IgM prevalence at ages 7 and 8.

The pet cats tested represent a subpopulation of Belgian cats that are most likely kept in excellent conditions and have restricted or no outdoor access. It can be assumed that the likelihood that these cats have been exposed to oocysts either in the environment or through hunting for prey is significantly lower than would be the case for free-ranging house cats or stray cats. Most Belgian house cats have a life between that of the no-outdoor access situation and stray cat situation. It can be expected that these cats have a higher seroconversion rate at younger age and a higher overall seroprevalence than the cats in this study. The tested pet cats are the most relevant subpopulation for assessing the risk of direct oocyst transmission to humans, since they live in close proximity to the owner and are most likely to defecate in an indoor litter box. Recently it has been shown that environmental oocyst contamination is concentrated in and around cat defecation sites (Afonso et al., 2008). Infection of the cat with T. gondii leads to the daily shedding of millions of oocysts in the feces over a period of 2-3 weeks pi (Bartova et al., 2003). When infection occurs via tissue cysts, the prepatent period is only a few days, but it is longer when the infection occurs via oocysts (Dubey and Frenkel, 1976; Dubey, 1996, 2001, 2005). About 2-5 days after fecal excretion, the oocysts in the feces have sporulated and can then remain infectious for several years (Dubey, 1998). Many studies have shown that experimentally infected cats no longer shed oocysts upon second infection with T. gondii, up to more than one year after primary infection (Frenkel and Smith, 1982a; Chavkin et al., 1994; Davis and Dubey, 1995; Lappin et al., 1996; Freyre et al., 2007). However, this immunity is not lasting, as 44% of T. gondii seropositive cats that had been infected 6 years previously were susceptible for oocyst shedding upon re-infection (Dubey, 1995). No evidence is available that would indicate whether naturally infected cats will reshed Toxoplasma oocysts later in life.

Prevention of oocyst shedding has been obtained with experimental vaccines, (Frenkel *et al.*, 1991; Garcia *et al.*, 2007), by the addition of 0.02% monensin to cat food (Frenkel and Smith, 1982b) or by antibiotic treatment a few days after infection (Sheffield and Melton, 1976; Freyre *et al.*, 2007). In contrast to infection with *Isospora felis*, infection with Feline Immunodeficiency Virus or Feline Leukemia Virus does not seem to provoke oocyst re-shedding in chronically infected cats (Dubey, 1976; Lappin *et al.*, 1992; Lappin *et al.*, 1996; Svobodova *et al.*, 1998).

By evaluation of the anti-Toxoplasma immunoglobulin isotypes, one can estimate whether the cat has been infected with T. gondii 1-3 weeks before (IgM+), 2-12 weeks before (IgM+ IgG+) or more than 3 months (IgG+) before serological testing (Lappin, 1996). By the time Toxoplasma IgG antibodies are detected, the oocyst shedding period has been completed. The short duration of the oocyst shedding period and the failure of most cats to repeat oocyst shedding on re-exposure explain why oocysts are rarely detected in the feces of naturally exposed seropositive cats. Indeed, only 0.28-0.9% of domestic and stray cats have been shown to actively shed oocysts, irrespective of their immune status (Childs and Seegar, 1986; Svobodova et al., 1998; Dabritz et al., 2007; Schares et al., 2008). In a previous study in Belgian domestic cats, about 50% tested positive for T. gondii specific antibodies, with 2.7% of cats aged 0-2 years actively shedding oocysts, the most at age 1 (Van Beeck et al., 1985). It must be noted that T. gondii oocysts are difficult to differentiate from Hammondia hammondi oocysts and it is estimated that in more than half of the cases the oocysts actively shed by cats are T. gondii (Schares et al., 2008).

Veterinarians need to be able to estimate the risk of *T. gondii* infection in - and transmission by - cats because of the potential health consequences in pregnant

women and immunocompromised owners, and they should counsel these cat owners as suggested in Table 1. Women who are seropostive before pregnancy provide sterile protection against Toxoplasmosis to their unborn child, and are not required to take preventive hygienic measures. In Belgium, about 50% of the population is estimated to be infected with *T. gondii*, and thus 50% is still at risk for infection (Breugelmans *et al.*, 2004). Congenital toxoplasmosis occurs in 9 out of 10,000 pregnancies in Belgium (Breugelmans *et al.*, 2004).

The cat population in Belgium is quite dense, including 1,675,000 registered cats in 2000 (National Institute of Statistics, 2007). In 2004, 23.9% of households in Flanders had at least one cat, and in Brussels and Wallonia this figure was 27.3% and 29.9%, respectively (National Institute of Statistics, 2007). Although the chance of encountering a cat shedding oocyts is relatively small and most domestic cats bury their feces in litter, the stability and quantity of oocysts dispersed in the living area of the domestic cat could pose a long-term risk of exposure to its human co-habitants. The observed geographical spreading of Toxoplasmosis in pet cats may be related to differences in the cat's outdoor access and feeding habits by the owners. We show that in the northern part of Belgium (with the exception of Limburg), more than 80% of the cats are seronegative, and thus more cats are at risk for infection and active oocyst shedding than in the southern part of the country, where 65% of the cats are still susceptible to infection. As seroprevalence is higher in the south of the country and Limburg, higher environmental contamination may be present as well. Hypothetically, if pet cats have frequent outdoor access, then the observed differences in seroprevalence should reflect the level of contamination (oocysts or infected wildlife) in the environment. However, no information on the frequency of outdoor access was available for the pet cats in this study. In stray cats from the city of Ghent, about 70% were found to be seropositive for T. gondii (Dorny et al., 2002).

For seronegative humans, in particular pregnant women and immunocompromised patients, it is advisable to have their cat tested for toxoplasmosis in order to assess parasite transmission risk (Table 1). We suggest that for epidemiological studies, in the event the owner seroconverted during the period at risk, it is important to assess whether the cat did likewise, in order to be able to trace back the source of infection either to the cat or to the consumption of infected meat.

ACKNOWLEDGEMENTS

This study was supported by the Belgian Federal Public Service for Health, Food Chain Safety and the Environment. We also thank "Les Amis de l'Institut Pasteur de Bruxelles" a.s.b.l. for their support.

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