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Short communication

Prevalence of *Toxoplasma gondii* infection in Belgian house cats

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Abstract

Five hundred and sixty seven sera of healthy house cats aged 3 months to 7 years, were examined for the presence of anti-toxoplasma antibodies by indirect immunofluorescence assay and compared to SAG1 and TLA enzyme linked immunosorbent assays as alternative test. Twenty-five percent of cats tested positive for IgG and/or IgM. Seroprevalence increased with age from 2% below 12 months of age up to 44% at age 7. Sensitivities of SAG1 and TLA ELISA were 84.1% and 88.6%, respectively. Peak levels in seroprevalence were correlated to increased IgG titers in TLA ELISA. Our results suggest that *T. gondii* infections are common in house cats and that there is a high chance for a negative cat to seroconvert in its second life-year.

Keywords: *Toxoplasma gondii*; Zoonosis; Seroprevalence; Transmission; ELISA; Indirect immunofluorescence assay; SAG1; TLA; Titer; Antibody

1. Introduction

Toxoplasma gondii is an obligate intracellular parasite and infects almost all warm-blooded animals and humans (Tenter et al., 2000). Cats get infected by feeding on infected meat (prey or treat), or by ingestion of sporulated oocysts, resulting in an enteroepithelial sexual cycle that leads to the shedding of oocysts in the environment (Dubey, 1986). Those oocysts rapidly sporulate and stay infectious for more than a year (Dubey, 1998). *Toxoplasma* may lead to serious morbidity and mortality in seronegative pregnant women and immunocompromised patients (Montoya and Liesenfeld, 2004), and to chorioretinitis in otherwise healthy individuals (Gilbert et al., 2006). A recent European multi-centre study has indicated that infected meat is considered as the main risk for congenital toxoplasmosis (Cook et al., 2000). However, oocyst contamination plays an important role in transmission of this parasite as strict vegetarians become infected with *Toxoplasma* (Hall et al., 1999; Roghmann et al., 1999). *Toxoplasma* seroprevalence in humans has been associated with living in close proximity to *Toxoplasma* seropositive cats: children who had cats were more likely to be infected than children who had no cats (Pereira et al., 1992), and toxoplasma seropositivity in adults was associated with living in close proximity to seropositive cats (Sukthana et al., 2003). In Belgium, about 50% of the population is infected with *T. gondii* and congenital toxoplasmosis occurs in 9/10,000 pregnancies (Breugelmans et al., 2004). The cat population in Belgium is quite dense and about 26% of households has at least one cat (DGS, 2007).

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The aim of this study was to determine the prevalence of toxoplasma infection in house cats ranging from 3 months to 7 years of age. Different serological tests were used to evaluate antibody responses against *T. gondii* and to evaluate whether they could match the IIFA reference test for cats.

2. Materials and methods

2.1. Sera

Serum from healthy house cats aged 3 months to 7 years was obtained from the National Reference Laboratory for Rabies at the Pasteur Institute of Brussels, Scientific Institute for Public Health. These sera were originally submitted for titration of rabies antibodies in a virus-neutralisation assay, which requires the samples to be complement-inactivated at 56 °C for 30 min. This test has to be performed in accordance with the European pet travel scheme, to allow the owners to enter certain rabies-free countries with their pets. All sera were taken by private veterinary practitioners between 2004 and 2006, prior to the travel of the animal outside the Belgian territory, and were provided with birth date of the cat and date of serum sampling. Samples were stored at −20 °C. All animals were healthy at time of sampling. Per age group, 49–90 samples were selected.

2.2. Indirect immunofluorescence assay (IIFA)

Fifty microliter of each serum diluted 1/50 in phosphate-buffered saline (PBS) was applied on slides coated with formalin-treated tachyzoites from the RH strain (Toxo-Spot IF, Bio-Mérieux, France) and incubated for 30 min at 37 °C. Slides were washed with PBS and incubated for 30 min at 37 °C with 30 μl of fluorescein isothiocyanate conjugated goat-anti-cat IgM (μ)- or goat-anti-cat IgG (H+L) (KPL, Gaithersburg, MA) diluted 1/20 and 1/40 in PBS, respectively, and stained with Evans Blue as counter dye. 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 Toxoplasma negative and positive feline reference sera from our laboratory, according to Toxo-Spot IF guidelines. Under ultra-violet light, the surface of the tachyzoite is fluorescent green with positive serum, while for seronegative samples the tachyzoite will show a red background stain. The specificity and sensitivity of the Toxo-Spot IF test for IgG are 98.44% and 95.08%, respectively, while for IgM it is not detailed by the manufacturer.

2.3. Parasites and total lysate antigen

Total lysate antigen (TLA) was prepared from RH tachyzoites as described previously (Jongert et al., 2007).

2.4. Enzyme linked immunosorbent assays (ELISA)

SAG1 coated ELISA plates (Meddens Diagnostics BV, The Netherlands) were incubated with cat sera diluted 1/50 in PBS with 10% foetal calf serum (FCS). SAG1-specific IgG was detected using an horseradish peroxidase (HRP) conjugated anti-cat IgG diluted 1/10.000 in 10% FCS in PBS (AbD Serotec, Oxford, UK). Colorimetric reaction was done with 3, 3′, 5, 5′-tetramethylbenzidine liquid substrate system (Sigma, St. Louis, MO) and stopped with 0.1N H_2SO_4. Plates were read at 450 nm in a spectrophotometer (Bio-Rad, Hercules, CA). Sera were considered positive if the optical density (OD_{450}) exceeded the cut-off value (= mean $OD_{450} + 3\times$ Standard Deviation OD_{450}) from three reference Toxoplasma negative cat sera at dilution 1/50. Determination of the IgG endpoint titer against TLA was performed as described previously with some minor modifications (Jongert et al., 2007). Briefly, 96-well ELISA Maxisorp plates (Nunc, Roskilde, Denmark) were coated with 10 μg/ml TLA overnight at 4 °C. After blocking with 10% FCS in PBS for 2 h at 37 °C, IIFA positive cat sera were applied in twofold dilutions starting at a dilution of 1/50 and incubated overnight at 4 °C. ELISA plates were further treated as described above. Endpoint titers were defined as the highest dilution where the optical density (OD_{450}) exceeded the cut-off value calculated with the OD_{450} from two reference *Toxoplasma* negative cat sera at the same dilution.

2.5. Statistical analysis

Logistic regression was used to investigate the correlation between *Toxoplasma* seroprevalence and age using SPSS 13 software (SPSS, Chicago, IL), and the correlation between antibody endpoint titer and seroprevalence per age group was investigated by Chi-square test for trend. Titer distributions were evaluated for normality with Kolmogorov–Smirnov test.