A cross-sectional survey of anti-\textit{Toxoplasma gondii} antibodies in Jerusalem cats

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Abstract

A cross-sectional seroprevalence study of anti-\textit{Toxoplasma gondii} IgG antibodies was performed in Jerusalem domestic and stray cats. An enzyme linked immunosorbent assay (ELISA) immunodiagnostic technique was used and results analyzed according to specific variables. The overall seroprevalence was 16.8%. After an initial high seroprevalence of 29.7% in kittens after 1–10 days of age, average positive titers dropped to a low of 7.0% at ages 11–60 days. Thereafter, average positivity increased continuously and was observed in 50% of cats more than 5 years of age ($P = 0.0081$). \textit{Toxoplasma} seroprevalence was highest in the month of summer (25.1%, $P < 0.0001$); domestic indoor cats had a higher seropositivity (39.0%) than stray cats (14.2%, $P = 0.0004$). On average, cats from the mainly Arab inhabited areas of Jerusalem showed a higher positive seroprevalence to \textit{Toxoplasma} than Jewish inhabited areas (34.1% and 16.0%, respectively, $P = 0.0032$). There were no significant differences in positivity rates between sexes, and between rates of those with the presence/absence of clinical symptoms similar to those of the disease.

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1. Introduction

\textit{Toxoplasma gondii}, an obligatory intracellular parasite, is the cause of toxoplasmosis, which is considered to be a major zoonosis. On the worldwide scale, prevalence rates by
serological survey in pregnant women vary from 7% to 51.3%, and in women with abnormal pregnancies and abortions, the rates vary from 17.5% to 52.3% (Dubey and Beattie, 1988). Many epidemiological investigations on *T. gondii* infection have been made in both humans and animals (Asami et al., 1995; Dubey, 1973, 1995). Various Israeli human serological studies in different parts of the country have been published mainly in the last decade (Flatau et al., 1993; Franklin et al., 1993; Nishri et al., 1978, 1993; Raz et al., 1993) with prevalence rates between 20% and 56% depending on ethnicity, religion, age, as well as socio-economic status.

No information about the seroprevalence of *T. gondii* in the definitive host, the cat, has been published in Israel. However, numerous studies have been carried out elsewhere. In the USA, Lappin (1996) states that approximately 30% of cats are seropositive for the disease. In Japan, Maruyama et al. (1998) showed 8.7% positive seroprevalence against *T. gondii* in 471 pet cats. Seropositivity increased with age and no differences were found amongst different sexes. Another Japanese study by Nogami et al. (1998) observed an overall prevalence of 6% in domestic cats. Outdoor cats had a higher prevalence than house cats. In the Czech Republic, a study of IgG seroprevalence by Svobodova et al. (1997) found an overall prevalence of 61.3% in cats with hardly any IgM (0.28%). Results from Egypt revealed that stray and domestic cats had IgG seroprevalence to *Toxoplasma* by indirect hemagglutination assay in 18.5% and 12.5%, respectively and by indirect immunofluorescence assay in 20.7% and 15.6%, respectively (Abu-Zakham et al., 1989). A study undertaken in Beirut, Lebanon between 1980 and 1983, observed a positive seroprevalence in 78.1% of 324 cats (Deeb et al., 1985). In Jordan, Morsy et al. (1980) found 18% of cats infected; Rifaat et al. (1976) found *T. gondii* antibodies in 39.6% of Egyptian cats using Sabin Feldman dye test methods. In Saudi Arabia, Hossain et al. (1986) found 30.3% of cats infected by serological methods. The purpose of this study is to analyze seroprevalence of *T. gondii* infection in cats residing in Jerusalem, Israel, where a large feral cat population among a relatively concentrated human population results in almost ideal conditions for infection to occur.

2. Materials and methods

Blood from 1062 cats representing mainly stray animals from the Jerusalem Municipality Animal Shelter, was withdrawn by intravenous or intracardiac methods into sterile 2.5 cc sterile syringes after intramuscular injection of animals with a Xylazine (1 mg/kg, Rompun)–Ketamine (10 mg/kg, Ketalar) combination. Alternatively, young kittens were euthanised using intraperitoneal administration of Pentobarbitone (Pental). These methods of euthanasia are approved by the American Veterinary Medical Association Panel of Euthanasia and Institutional Animal Care and Use Committee (Anon., 2001, updated in 2001). Blood was clotted, centrifuged, and the serum collected into coded eppendorf microtubes. They were stored at −20 °C until further examination. Data such as age, habitat, sex, area of capture, clinical symptoms, and date of blood collection was noted. Samples were systematically selected from a calendar year’s collection of data from April 1999 to April 2000 and tested by the enzyme linked immunosorbent assay method for IgG antibody.
Two local veterinary laboratories conducted systematic sampling of domesticated cats from June 1999 and supplied the sera for this part of the study. The ELISA test was chosen because of the ease of its application and because of a superior sensitivity and specificity in detection of *T. gondii* specific IgG when compared with latex agglutination and indirect haemagglutination tests (Lappin and Powell, 1991).

2.1. Categorization of groups and operational definitions

Age was categorized into: 1–10 days, 11–60 days, 61–365 days, 366–730 days, 731 days to 5 years, and greater than 5 years of age. Age in stray cats were determined by means of (1) dental eruption (approximately at 2 weeks of age when first deciduous incisors erupt; at 2 months of age when upper deciduous premolar two erupts—as described in the Merck Veterinary Manual), (2) level of calculus (positively associated with increased age), (3) opening of eyes (at 10–14 days), and (4) size of the animal (increasing up until about 6 months of age). Exact ages of cats were noted when previous history was known. Cats were defined as stray when no previous knowledge of ownership and no physical clues as to care, such as collars, castration or ovariohysterectomy scars were available.

Level of domestication was categorized as: entirely housebound (indoors), in/outdoor cat, and entirely outdoors as determined by questioning of the presenting owner of the animal.

Area of capture was divided into geographical areas of Jerusalem that are predominantly inhabited by Arab Israelis or by Jewish Israelis.

Cats or kittens presenting with *Toxoplasma* like symptoms, such as vomiting or diarrhoea, upper respiratory signs (conjunctivitis, rhinitis, sneezing, nasal discharge, buccal ulceration, dyspnoea), ophthalmic symptoms (uveitis, corneal ulcers, conjunctivitis), neurological signs including degrees of paralysis, and emaciation were regarded as clinically positive.

2.2. Serology

2.2.1. Antigen preparation

Soluble *Toxoplasma gondii* antigen of the RH strain (Capricorn Labs, Scarborough, ME, USA) derived from intraperitoneal exudates of infected mice was sonicated using a microtip for three 10 s pulsations with a 20-s rest period by Ultrasonic, Formingdale, NY and at number four setting. Protein concentration of about 7.7 mg/ml was confirmed by the modified Lowry method (Lowry et al., 1951). Antigen was diluted to 5 mg/ml, aliquoted, and stored at −70 °C until further use. Protein A of 0.1 μg/ml conjugated to horseradish peroxidase (Immunopure Recomb, Pierce Laboratories, Rockford, IL, USA) in the wash buffer solution was used as secondary antibody. After performing initial checkerboard analyses to determine the ideal concentration of antigen, serum, and secondary antibody, it was decided that an antigen concentration of 0.75 μg protein/well and dilutions of both 1:80 and 1:160 sera were best for test purposes. Secondary antibody was used at a dilution of 1:10,000.
2.2.2. **ELISA (enzyme linked immunosorbent assay)**

Plates, comprised of 96 wells, were filled with 50 μl of 0.1 M bicarbonate buffer (pH 9.0) and 0.75 μg of antigen per well, the day before testing was performed to coat the wells. The following day the plates were washed three times with a phosphate-buffered saline solution (20 mM sodium phosphate and 150 mM NaCl)/0.05% Tween-20 (pH 7.4) and then blocked by 100 μl PBS/Tween/1% bovine serum albumen per well. Incubation of the plates at 37 °C for 1 h followed. The plates were washed as above and 100 μl of an appropriate serum dilution in PBS/TWEEN per well was added in triplicate. Each plate contained a negative and positive sera control in triplicate.

After another hour at 37 °C incubation, the wells were washed three times before 50 μl of Protein A-HRP (1:10,000) was added and the plates incubated for an hour at 37 °C. After three washes, 100 μl/well of phosphate-citrate buffer-substrate solution with sodium perborate/2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) (Sigma–Aldrich labs, Rehovot, Israel), prepared according to their recommendations was added to each well and incubated for 30 min at room temperature. Absorbances (optical density (OD)) were measured with an Elx-Universal Microplate Reader, Winooski, VT at 405 nm wavelength. On all occasions, testing was done by the same individual and according to the above strictly adhered to protocol. At least 31 previously recorded positive and negative sera were retested using different reagent aliquots to test repeatability. Results were based on (1) 1:80 titer for IgG antibodies to T. gondii; (2) 1:160 titer; or (3) negative (0) defined as a titer less than 1:80. Samples were regarded as positive if their mean absorbance at each titer was three or more times the mean absorbance of the negative control at the same titer.

Blood samples were never taken later than 10 days of initial cat housing.

2.3. **Statistical analysis**

Statistical analysis was performed by multiple logistic regression and Pearson’s chi-square. Significance was measured with a two–tailed test where $P < 0.05$. The SPSS computer package was used for analysis and graphics of results.

3. **Results**

The study represented a calendar year’s sample of 1062 cats comprised of 463 males (43.6%), 596 females (56.1%); 41 domestic indoor cats (3.9%), 35 domestic outdoor cats (3.3%), 52 domestic in/outdoor cats (4.9%), and 934 stray cats (87.9%).

Forty-four (4.1%) cats were from Arab inhabited Jerusalem and 1018 (95.9%) from Jewish inhabited areas of Jerusalem. Fewer cats were sampled from east Jerusalem as the Arab Moslem and Christian residents generally complain less to the Municipal services about stray cats.

Clinical symptoms are presented in 169 (15.9%) of cats and 893 (84.1%) had no external symptomology as defined earlier in the operational definition. Ages of cats were from 1 day to 18 years old. The number of cats bled in winter were 190 (17.9%), 341 (32.1%) bled in spring, 358 (33.7%) bled in summer, and 173 (16.3%) bled in autumn. In
all variables except sex, where four missing cases existed, data was complete. Fig. 1 shows percentage differences in seropositivity amongst varying age groups. An initial high positive seroprevalence in young kittens under 10 days of age is seen, after which a sudden drop in seropositivity in kittens 11–60 days of age occurs. Then a gradual rise in positive seroprevalence is seen according to increasing age until a high of 50.0% in cats estimated to be over 5 years of age prevails ($P < 0.001$). Fig. 2 shows the variation of positive seroprevalence according to cat lifestyle. Interestingly, domestic indoor and in/outdoor cats show the highest positive seroprevalence followed by domestic outdoor and lastly stray cats. Fig. 3 depicts seroprevalence according to area of cat capture. Cats of mainly Arab inhabited east and north Jerusalem (34.1%) had significantly higher seropositive results as compared to cats in mainly Jewish inhabited parts of Jerusalem (16.0%, $P < 0.006$). Fig. 4 shows seropositivity according to season of blood collection. A highly significant difference in infection rates ($P < 0.001$) is seen with a change of season with highest titers seen in summer followed by spring, autumn, and winter. Differences in seropositivity between sexes were not significant ($P = 0.880$) as well as differences between cats presenting with clinical symptoms and those without ($P = 0.572$).

Using multiple logistic regression in order to attempt to control for the possible confounding of other independent variables, cats of Arab east Jerusalem areas were shown to have significantly higher *Toxoplasma* seropositivity (34.1%) than cats from Jewish west Jerusalem (16.0%, $P = 0.0032$; odds ratio = 2.7681).

Fig. 1. 3D column diagram depicting age vs. percentage seropositivity ($P < 0.001$).
4. Discussion

The ELISA method was used due to the ease of its preparation and application as well as
due to its high sensitivity and specificity when compared with other test methods (Lappin
and Powell, 1991). The test was reliable as seen by the results obtained on re-examination
of 31 samples of the same sera on different days and with different reagents. When eight
samples were tested by ELISA and compared with a commercial Immunocomb ELISA
(Biogal, Israel), excellent repeatability of the results were observed. Also, 45 samples were
tested by ELISA and compared to a commercially available test kit based on the modified
agglutination assay (MAT, Toxo-Screen DA, bio-Merieux, France). The MAT sera was
screened at titers greater or equal to titers of 1:40. In each case, both tests correlated well
with degree of agreement 100%. Also noted were identical results obtained when kittens
within individual litters and their mothers were tested. This was demonstrated in nursing
seropositive female cats and their kittens younger than 10 days. For example, within the
results of sera from a female one-year-old cat, C53 and its kittens 6 days of age, K154
(male), K155 (male), and K156 (female), all tested seropositive at titer 1:160. Likewise, a
2-year-old female cat, C63, as well as its 2-week-old kittens, K189 (female), K190 (male),
and K191 (female) were all seronegative. This confirms colostral transfer of IgG immunoglobulins to the young from the female cat as previously reported (Omata et al., 1994).

In young cats, titers dropped and only increased significantly from 60 days of age, which is about the age when kittens begin to hunt (Dubey, 1986) and possibly ingest oocysts or bradyzoites from cat stools or infected prey. Thereafter, seropositivity was positively associated with age as seen in Fig. 1, until 18 years of age (representing the oldest cat of known age that was screened). These results are similar to those observed by Furuya et al. (1993), Margileth (1968), Maruyama et al. (1998), and Rifaat et al. (1976). This may demonstrate continuous exposure to antigen and thus boosting of the immune system with age. It should be mentioned that although the method for aging of cats appeared to be adequate for kittens until the age of 5–6 months, the method proved to be lacking for approximation of age in older cats. The majority of cats that tested positive had relatively high titers (1:160) or more.

Fig. 3. 3D column diagram showing area of capture vs. seropositivity ($P < 0.006$).
Surprisingly, domestic indoor cats showed a higher seropositivity than any other group, which is unlikely to have occurred by means of exposure to other oocysts or bradyzoite infection from the environment. However this same group was older and therefore, may have been confounded by age or the owner history may have been unreliable because of recall bias or other unknown reasons. Table 1 shows age distribution of cats according to stray or domestic lifestyle.

Note that in cats over 5 years old, 82.6% of the sample group were of domestic lifestyle whilst only 17.3% are stray cats. Similarly, in the younger age groups under 1 year of age, 84.5–99.6% were stray cats in the relevant categories. Thus, our assumption of confounding is strengthened. Due to categories with few samples, logistic regression could not control fully for confounding.

Other researchers observed quite the opposite (Knaus and Fehler, 1989). In fact, one could expect a greater exposure of feral cats to cysts from infected wildlife. Thus confounding of this variable may have occurred reducing this category’s overall positivity. West Jerusalem had significantly \((P = 0.002)\) less positive cats (16%) as compared with cats from Arab east and North Jerusalem (34.1%). This may demonstrate the greater likelihood of exposure of cats in these areas to *Toxoplasma* by zoite ingestion due to their reliance on small mammals and birds for nutrition. Seasonal seropositivity was highest in summer and lowest in the winter months. Usually, IgG titers higher than 1:160 would take between 2
and 4 weeks to develop (Lappin et al., 1995). Thus, exposure probably occurred mainly in the late months of spring and early summer months that are wetter, cooler, and more abundant with available prey for cats as compared to drier and hotter months in late summer. Results were similar in studies by Rifaat et al. (1976) and Katsube et al. (1972). Rifaat et al. (1976) also observed no significant differences in seropositivity between sexes \( (P = 0.894) \) and between those with/without clinical symptoms \( (P = 0.574) \).

### 5. Concluding remarks

A similar cumulative toxoplasmosis seroprevalence in cats was observed in our study as compared to other regional surveys, notably, Jordan, Saudi Arabia and Egypt. Although other researchers have reported different findings (Knaus and Fehler, 1989), a higher seroprevalence in domestic compared to stray cats was observed in this study. This may have important implications for the risk of infection in cat owners. Studies have, however, shown inconclusive results as to the risk potential of infection for the owners of cats, which have a previously known positive Toxoplasma antibody titer (Dubey, 1986). IgG antibody titers in cats exposed to T. gondii have been seen to rise and remain high up to a number of years after infection (Lappin et al., 1995). This is important before drawing any major conclusions as to the relevance of differences seen amongst different variables, especially taking into account that only medium range titers were measured and also due to the cross-sectional nature of this study.

Pitfalls in the study were: (1) a lack of an effective aging system for stray cats; (2) a trichotomous dependent variable which lacked the ability for quantitative titer measurement; and (3) the lowest titer of 1:80 that was used for positively infected cats (this was lower than the 1:64 titer used by Lappin in his ELISA studies and the 1:32 titers or less used by other investigators using other immunodiagnostic methods).

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References


