RESEARCH ARTICLE
JIVA 21 (2) August 2023
https://doi.org/10.55296/JIVA/21.2.2023.39-44

Received on 17/02/2023
Accepted on 19/06/2023

SEROLOGICAL SCREENING FOR TOXOPLASMOSIS IN DOMESTIC CATS OF CENTRAL KERALA

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ABSTRACT

The present study was designed to record the seropositivity rate of toxoplasmosis in domestic cats presented to the University Veterinary hospitals. Serum samples were collected from 43 cats and subjected to an indirect enzyme-linked immunosorbent assay (ELISA) for detection of IgG antibodies against *Toxoplasma gondii* using a commercially available kit. Out of 43 cats tested, 12 showed a positive reaction for antibodies against T. gondii (27.90 %). The rate of seropositivity was higher in cats above 3 years, male cats and those with outdoor access. But a statistically significant association was observed only between sex of cats and seropositivity rate whereas no significant association has been observed with age and rearing practice. The identification of seropositive cats in a community is important to estimate soil contamination with oocysts and determine populations with a high risk of exposure to *T. gondii.* The results of the present study stress on the need for establishing prevention strategies to minimize this neglected zoonosis.

Keywords*:* Domestic cats*, Toxoplasma gondii*. seropositivity ELISA

INTRODUCTION

Toxoplasmosis caused by *Toxoplasma gondii,* is one of the most common parasitic zoonosis world-wide and infects a wide range of warm-blooded vertebrates, including humans. It is transmitted most commonly by the ingestion of tissue cysts in raw or undercooked meat. Infection also occurs by consumption of food or water contaminated with sporulated *T. gondii* oocysts. Cats are the definitive hosts and play an important role in the life cycle of *T. gondii*. They facilitate the genetic recombination between strains, as well as environmental contamination (Vilares *et al*., 2014). Cats can excrete millions of oocysts through faeces after primary infections. These oocysts are

highly resistant in the environment, surviving for months in soil and water and after sporulation are infective for animals and humans (Dubey, 1995).

The identification of seropositive cats in a community is important to estimate the environmental contamination and determine the populations with a high risk of exposure to *T. gondii.* Since cats are one of the most popular pet animals, their infections pose zoonotic significance in the human population. The present study was designed to find out prevalence of *T.gondii* antibodies among domestic cats in Thrissur district of Kerala.

MATERIALS AND METHODS

Domestic cats presented to the University Veterinary hospitals, Mannuthy and Kokkalai during the period of 2016-17 were screened for antibodies against *T. gondii*. Serum samples were collected from 43 cats and subjected to an indirect enzyme-linked immunosorbent assay (ELISA) for the detection of IgG antibodies against *T. gondii* (Multi-species ID Screen® Toxoplasmosis Indirect, IDVET, France).

Test serum samples (10µl) and positive and negative controls were diluted to 1:10 using 90 µl of dilution buffer supplied with the kit. The diluted serum samples were transferred to an ELISA microplate coated with *P30* antigen of *T. gondii* and the plate was incubated at 21ºC (± 5ºC) for 45 minutes. The wells were emptied and washed three times with 300 µl of 1X wash solution using an ELISA plate washer. Added hundred µl of 1X conjugate to each well and incubated at 21 ºC (±5 ºC) for 30 minutes. The wells were emptied and washing procedure was repeated as mentioned above. Hundred microlitre of substrate solution was added to each well. The plate was then incubated in the dark at 21 ºC (±5 ºC) for 15 minutes. Hundred microlitre of stop solution was added to each well in order to stop the reaction. Optical densities were measured at 450 nm using an ELISA plate reader (Varioskan Flash, Thermo Fisher Scientific) and SkanIt Software 2.4.5 RE. The test was considered valid after it satisfied the following conditions; The mean value of the positive control Optical density ($O.D._{PC}$) was greater than 0.350 and the ratio of the mean O.D. values of the positive and negative controls is greater than three ($O.D._{PC}$/ $O.D._{NC}$> 3). For each sample, the seropositivity (S/P) percentage was calculated as follows: S/P per cent = ($O.D._{sample}$- $O.D._{NC}$ / $O.D._{PC}$ - $O.D._{NC}$) x 100.The result was considered as negative when S/P % ≤ 40 per cent, Doubtful when 40 < S/P < 50 and positive when S/P ≥ 50 per cent.

RESULTS AND DISCUSSION

Out of the 43 serum samples tested, 12 were positive for antibodies against *T. gondii* and thus, seropositivity was 27.90%. The seropositivity ranged from 50 to 224% in positive cats. Varying levels of seroprevalence has been reported among cats from different parts of world (Zhang *et al*., 2010; Montazeri *et al*., 2020). Seroprevalence of *T. gondii* in domestic cats was estimated to be 30 to 40% worldwide (Dubey and beattie 2010). Montazeri *et al*. (2020) reported a higher seroprevalence among domestic cats in Australia (52%) and Africa (51%) and a lower prevalence in Asia (27%). Hatam-Nahavandi *et al*. (2021) also reported a lower prevalence in Asia (28.3%). The results of the present study are in agreement with these observations. A lower prevalence when compared to the present study was reported in serosurveys conducted at Bagkok Metropolitan region (6.5%) by Inpankaew *et al*. (2021) and the Netherlands (18.2%) by Opsteegh *et al*. (2012). The variations in the seroprevalence of toxoplasmosis in different regions might be due to geographical factors or feeding and animal welfare conditions for cats in these areas.

The seropositivity of *T.gondii* in various age groups and sex of cats and under different rearing practice is depicted in Table1.The rate of seropositivity was higher in cats above three years (37.5%) and lowest in kittens below one year age (18.18%). But as per Fisher's exact test, no significant (p>0.05) association could be observed between age and prevalence of *T.gondii* antibodies. The higher seropositivity has been reported with increasing age of the cats (Ahmad *et al*, 2014; Must *et al*., 2015) indicating that age is a risk factor for seropositivity in cats. Higher seropositivity in older cats might be related to increased exposure to *T. gondii* oocysts through food, water and outdoor activities (Xia *et al.,* 2022).

Table 1. Seroprevalence of Toxoplasmosis in domestic cats

Risk factor	Number tested	Number positive	Per cent
Age			
Below 1 year	11	2	18.18
1-3 years	24	7	29.17
Above 3 years	8	3	37.50
Sex			
Male	23	11	47.82**
Female	20	1	5.00
Rearing practice			
Indoor	15	3	20.00
Outdoor access	28	9	32.14

** Significant (p<0.01)

The overall seropositivity of *T. gondii* infection was more in males (47.82%) than females (5%) and as per Fisher's exact test, there was significant ($p<0.01$) association between sex and seroprevalence of *T.gondii*. This result is in agreement with previous reports from Norway (Saevik *et al*., 2015). But no significant difference in the seroprevalence of *T.gondii* could be detected in male and female cats in Japan (Nogami *et al*., 1998), Brazil (Cardia *et al*., 2013), China (Xia *et al*., 2022) and Saudi Arabia (Mohammed *et al*., 2019). On the other hand, a higher seroprevalence of *T. gondii* has been reported in female cats in Hungary (Hornok *et al*., 2008).

The seropositivity rate was higher in cats with outdoor access (32.14%) than those kept indoors (20%). But as per Fisher's exact test, there was no significant ($p>0.05$) association between rearing practice and seropositivity in the present study. Similar observations were also reported by Dubey *et al*. (2002) in rural Ohio and higher *T. gondii* seroprevalence found in cats with outdoor access has been attributed to their carnivorous behaviour and eating prey animals such as rodents and birds. Opsteegh *et al*. (2012) identified hunting behaviour and feeding of raw meat as the risk factors for higher prevalence of toxoplasmosis in stray cats, which could be a potential target for intervention measures to prevent infections.

Currently there is an increase in the number of pet cats in Kerala. Since cats are one of the most popular pet animals, their infections may affect their owners and others living in their environment. The results of the present study stress on the need for establishing prevention strategies to minimize the occurrence of this neglected zoonosis in central Kerala. The proper disposal of cat litter, keeping cats indoors to minimize their acquisition of infection from prey or the environment, and reducing the feral cat population are the recommended measures. Also further research is warranted to assess the prevalence in other parts of the state and to investigate the links between cat ownership and human *T. gondii* infection.

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