Prevalence of *Toxoplasma* antibodies by using modified direct agglutination test in dogs in Chennai

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Abstract

Serum samples collected from 400 dogs in and around Chennai, India were analysed for antibodies to *Toxoplasma gondii* by modified direct agglutination test (MDAT) and the seroprevalence was recorded as 29.25%. The titre value of 1:64 and above was considered as positive. Statistically there was no significant correlation between age and sex-wise occurrence of toxoplasmosis. Non-descript dogs showed higher seropositivity than other breeds.

Keywords: Dogs, Modified direct agglutination test, Seroprevalence, *Toxoplasma gondii*.

Introduction

Toxoplasmosis, a widespread emerging zoonotic disease, caused by *Toxoplasma gondii*, an obligate intracellular coccidian parasite, affecting a wide range of animals including man. The disease is characterized by subclinical infections in the hosts except in immunocompromised patients. Toxoplasmosis is an economically important zoonotic disease transmissible to people from pets (Dubey, 1985). In most companion animals, the clinical symptoms of toxoplasmosis are confined to respiratory distress, pneumonia, enteritis, limited joint mobility, lymphadenopathy, ascities, abortion, stillbirth, chorioretinitis, blindness and uveitis (Ahmed et al., 1983). The incidence of toxoplasmosis shows a highly variable distribution pattern from country to country and ranges from 0-90 per cent (Dubey and Beattie, 1988). In India Dubey, (1987) recorded 24.5 per cent seroprevalence of toxoplasmosis in dogs during 1970 to 1986. The objectives of the present study was to assess the seroprevalence and discuss the potential risk factors involved in canine toxoplasmosis.

Materials and Methods

Blood samples: Blood samples (2 ml) were collected aseptically from 250 dogs from Madras Veterinary College Teaching Hospital, 75 dogs from private veterinary hospitals, Chennai and 75 dogs from Government Veterinary Hospital, Saidapet, Chennai. During collection, the history on age, sex, breed, clinical symptoms and potential risk factors were noted. The serum samples were separated and preserved in sterile 2 ml storage vials at -20°C until further use.

*Reference sera:* positive and negative control sera samples were procured from Dr. David Buxton, Moredun Research Institute, International Research Centre, UK and stored at -20°C until use.

*Preparation of antigens:* Modified direct agglutination test antigen (MDAT antigen) was prepared as per the procedure of Vijaya Bharathi et al. (2003). *Toxoplasma gondii* RH strain was obtained from the Department of Veterinary Parasitology, Veterinary College, Tirupathi. This isolate was propagated in mouse peritoneal fluid. The peritoneal fluid of terminally infected mice was harvested after 48-72 hours of inoculation of *T. gondii* RH strain into the peritoneal cavity and centrifuged at 2000 g for 5 min to sediment the tachyzoites. The tachyzoites were resuspended in 3-4 ml of phosphate buffered saline (PBS), pH 7.2 and the cell suspension was layered over 6 ml of Histopaque (Sigma, USA) and again centrifuged at 2000 g for 5 min. The layer containing tachyzoites was carefully aspirated using a Pasteur pipette taking care to remove all the parasites. The tachyzoites were resuspended and washed thrice in PBS, pH 7.2. After third washing, the tachyzoites were suspended in 6% formaldehyde solution in PBS (pH 7.2) and incubated for 20 hr. The suspension was centrifuged at 2000 g for 5
The tachyzoites were finally resuspended in PBS and stored in sterile vials at 4°C. A drop of the suspension was examined under high power to ascertain whether the tachyzoites retain their normal crescentic shape.

**Test procedure:** Modified direct agglutination test was done as per the procedure described by Desmonts and Remington (1980). Double fold dilutions of serum samples from 1:2 to 1:4,096 were tested. To avoid interpreting non-specific reactions as positive results, only titres of 1:64 or higher were considered as positive for MDAT.

**Results and Discussion**

Of 400 dog sera samples screened for the presence of antibodies against *T. gondii* 117 (29.25%) were positive. Antibody titres ranged from 1 in 64 to 1 in 4096. Out of the 117 positive samples, 37 (31.62%) had a titre of 1 in 64, 17 (14.52%) had a titre of 1 in 128, 24 (20.51%) had a titre of 1 in 256, 23 (19.65%) had a titre of 1 in 512, 9 (7.69%) had a titre of 1 in 1024, 5 (4.27%) had a titre of 1 in 2048 and 2 (1.7%) had a titre of 1 in 4096.

Dubey and Thulliez (1989) reported that MDAT was more suitable, reliable and sensitive test in detection of antibodies against *T. gondii*.

Chhabra et al. (1985) reported 34.8% of dogs in Ludhiana and 26.50% of dogs in Hisar as seroreactors for *T. gondii* antibodies. Chellappa (1987) and Sharma and Gautam (1974) reported comparatively low sero-positivity of 14.5% in Chennai and 13.8% in North India respectively. Mineo et al. (2004) reported a high seroprevalence of 30.3%, 91.2%, and 5.7 per cent of dogs from Veterinary hospitals, private veterinary clinics and stray dogs respectively in Brazil.

**Sex-wise occurrence of toxoplasmosis:** Of 117 cases by modified direct agglutination test, 60/117 (51.28%) males and 57/117 (48.71%) females were positive for *T. gondii* antibodies. Statistically no significant sex-wise difference (P>0.05) could be detected in prevalence of *T. gondii* in dogs. Similar observations were made by Franco et al. (2004), who found that the seropositivity of *T. gondii* was 76.4% and the association between the prevalence and the variables namely sex, age, type of food showed that there was no association between sex and *T. gondii* antibodies.

**Age-wise occurrence of toxoplasmosis:** Age-wise grouping of the 117 dog serum samples were done. Accordingly the animals were grouped as less than 2 years, 2-4 years, 4-6 years, 6-9 years and above 9 years. The seropositivity were 24.78% (29/117), 17.09% (20/117), 24.78% (29/117), 11.11% (13/117) and 22.22% (26/117), respectively. Higher seropositivity was noticed in the age group of less than 2 years, 4-6 years and more than 9 years. However statistically there was no significant correlation between different age groups with prevalence of *Toxoplasma* infection in dogs in Chennai city.

In contrast, Franco et al. (2004) observed that the seropositivity in dogs over 24 months (85.5%) compared with 50% in dogs less than 24 months and concluded prevalence of toxoplasmosis tends to increase with age. But in the present investigation the highest seroprevalence reported was in young and old animals.

**Breed-wise occurrence of toxoplasmosis:** Breed-wise distributions of the 117 seropositive dog serum samples were done. The highest seropositivity was observed in non-descript groups (33.33%) followed by Spitz (18.80%) and German shepherd (15.38% and the lowest seropositivity was notice in rare breeds (8.54%).

Dubey et al. (2003) reported *T. gondii* antibodies in Great Dane breed of dogs in Brazil. The increased positivity of *T. gondii* infection in non-descript dogs may be due to large number of samples examined and probability of ingesting uncooked meat and exposure to the oocyst from the environment.

**Clinical epidemiology of toxoplasmosis:** Symptom-wise occurrences of the 117 seropositive dog serum samples were analysed. Skin disorders, ocular disorders, neurological disorders, concurrent infectious diseases, abortion and non-specific symptoms were observed. The high seropositivity was noticed in 22.22% (26/117) of those with ocular disorders, followed by 19.65% (23/117) of those with concurrent infectious diseases.

Dedyurin et al. (1962) reported signs of paralysis of limbs, catarrh of the upper respiratory tract, pneumonia, eczema, stillbirths and neonatal death in dogs. Netrebko et al. (1965) recorded the signs of vomiting, diarrhoea, emaciation, sialorrhoea, thirst, anorexia, stomatitis, limbs, catarrh of the upper respiratory tract, pneumonia, eczema, stillbirths and neonatal death in dogs. Novinskaya (1965) observed digestive and central nervous system disturbance in *Toxoplasma* infected dogs. Chellappa (1987) found that, stillbirth, dermatitis, digestive and nervous disorders were seen in the *Toxoplasma* seropositive cases.

In the present study, 82.35% (14/17) of canine distemper had antibodies to *T. gondii*. This study coincided with Campbell et al. (1955) also recorded that 99% of dogs infected with distemper virus infection had *T. gondii* antibodies. Ahmed et al (1983) reported that association between infections with *T. gondii* and other disease conditions (canine distemper, pneumonia, tumors) in dogs.

Ahmed et al. (1983) investigated that *T. gondii* antibodies in the sera of dogs associated with stress, joint
concluded that urban areas. cats could be indirect indicator of the parasite spreading in oocysts from the environment. Meireles the higher probability of dogs ingesting tissues of animals or higher incidence of stray and owned dogs had antibodies against respectively. Among the urban dogs, 31.6% and 5.2% of in the rural and urban dogs was 34.3% and 19.7%, respectively. Ahmed, B.A., Gaafar, S.M., Weirich, W.E. and Kanitz, C.L., references

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