SEROLOGICAL SURVEY OF *TOXOPLASMA GONDII* IN DOGS AND CATS

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ABSTRACT

A serological survey for *Toxoplasma gondii* in dogs (n=40) and cats (n=10) was conducted by using a Latex agglutination test (LAT). The overall seroprevalence of *T. gondii* in canines was 50%. Out of total 9 dogs were found seropositive at 1:256, giving an evidence of presence of infection. The seroprevalence of *T. gondii* in canines was inversely related to the age i.e., 52% at 6 months and 33.33% at 4 years of age. Little variation in seropositivity was observed between males (57.89%) and females (42.85%) or between exotic (46.15%) and local (57.14%) breeds. However, tremendous variation in seropositivity was found between stray dogs (78.57%) and pet dogs (34.61%) and between dogs having close contact with cats (50%) and without contact (16.16%). Out of the tested bitches, 66.66% were seropositive, mostly at 1:16 indicating residual immunity. The overall seroprevalence of *T. gondii* in cats was 60%, three at screening dilution of 1:256, suggesting recent exposure to *Toxoplasma*. The seroprevalence of *T.gondii* in cats was directly related to age. A significant difference in seropositivity was observed between stray cats (66.66%) and indoor cats (57.14%) and between females (70%) and males (40%).The seropositive rate in local breeds of cats was high (66.66%) as compared with exotic (50%). This test might give false positive results due to interfering factors (rheumatoid factor and IgG class antibodies). So it is not a "Gold standard" test for the concrete diagnosis of toxoplasmosis.

Key Words: Serological survey, *Toxoplasma gondii*, dogs, cats, Latex agglutination test.

INTRODUCTION

Toxoplasma gondii, an intracellular protozoan was first discovered by Nicolle and Manceaux in 1908 in the North African rodents. *T. gondii* is crescent or banana-shaped organism and may appear intracellularly in peripheral blood smear (Coles, 1980). The organism has been reported to infect herbivores, carnivores and omnivores by various workers from different parts of the world. Aganga *et al.* (1981) have reported the prevalence of *T. gondii* antibodies in food producing animals. Felines being definitive host, play a pivotal role in the epidemiology of toxoplasmosis, excreting oocysts in their faeces (Rizwan *et al.*, 1999) which can survive for several months to year under severe environmental conditions, and are remarkably resistant to most disinfectants. Cat is the only host in which the organism undergoes sexual reproduction within the intestines.

The organism is cosmopolitan in distribution. Toxoplasmosis is an important zoonotic disease. It is estimated that over one third of the world population have contracted the infection (Kean, 1972).

After ingestion by any host, the organisms escape from its cysts and penetrate the intestinal wall and emerge either as tachyzoites or sporozoites. In an immunocompetent individual, the organism will encyst itself in the brain, muscles, and eyes as a bradyzoites (Burney, 1996). However, if the host becomes immunosuppressed, the organism may reactivate and cause disease.

T. gondii causes feline uveitis in association with feline leukemia virus (FeLv), Feline immunodeficiency virus (FIV) and Feline infectious peritonitis (FIP). Some cats, like AIDS patient develop encephalitis secondary to toxoplasmosis in chronic FIV infection (Burney, 1996).

T. gondii causes respiratory, alimentary and neurological disturbances in canines in conjunction with viral infection and stress factors. Above all, polymyositis-polyradiculitis due to *Toxoplasma* infection has been reported (Suter *et al.*, 1984).

Local information on canine and feline toxoplasmosis is not available. Furthermore, it is very laborious and tedious to obtain tissue for histopathology and cultivation of the organism. Therefore, at present serological testing is the most widely used as practical method for the diagnosis of *T. gondii*. The study was carried out to record the seroprevalence of toxoplasmosis in canines and felines.

MATERIALS AND METHODS

Collection of Samples

A total of 50 serum samples (dogs, n = 40 and cats n = 10) were collected from outdoor clinic, Department

of Clinical Medicine & Surgery, University of Agriculture, Faisalabad as well as from stray dogs and cats. History of the household dogs and cats was noted, especially with regards to their access to outside. The age of the stray dogs and cats was estimated on the basis of their general appearance, but the age of the household dogs and cats was obtained through the owners. All other relevant informations were recorded on proforma regularly.

Under aseptic measures, 6-8ml of blood was withdrawn by veinpuncture with the help of disposable syringes and was transferred to a screw capped sterile clean test tube slowly to avoid haemolysis.

Separation of Serum

All the blood samples were labeled with the species, outdoor registered number and the date of collection. The samples were left for about an hour for blood clotting to occur and were further processed as described by Samaha *et al.* (1993). Repeated freezing and thawing was avoided.

Analysis of the Serum Samples

All the serum samples were analyzed for specific IgG anti-toxoplasma antibodies using Latex Agglutination (LA) test. For this purpose, the commercial test kit used was "Toxoplasmosis Latex" manufactured by Quimica Clinica Aplicada, S.A. Amposta Spain.

Interpretation of Results

The following interpretations were made.

a. Negative 1:16 sera indicate absence of immunity
b. Positive 1:16 sera indicate residual or non specific immunity.
c. Positive titers from 1:32 to 1:128 are due to acquired or evolving immunity.
d. Positive titers equal or higher than 1:256 suggest possible recent contact.

It is recommended that a new determination should be done three weeks later in case 'd' and 'c'. Then a titer rise of at least 3 dilutions suggest a positive diagnosis.

Sero-prevalence

The data, thus obtained were analyzed at different angles by calculating the percentage seropositivity of toxoplasmosis in dogs and cats.

RESULTS AND DISCUSSION

The results of the serological findings of *T. gondii* in dogs and cats were summarized in Tables 1-7.

DISCUSSION

Seroprevalence of *T. gondii* in Canines

Toxoplasmosis is very important protozoan disease of canines. The current study revealed that the onset of the illness was marked by an insidious development of fever with lassitude, anorexia and diarrhoea; occasionally it may be sudden with vomiting followed by fits and paralysis and other neurological manifestation.

In the present study, the overall prevalence of anti-toxoplasma antibodies in canines was recorded as 50% (Table 1).

Table 1. Prevalence of anti-toxoplasma antibodies in stray and pet dogs by using LAT.

Dog Habit	No. Tested	No. of dogs Antibody titers (reciprocal) 16	128	256	Sero-Positive No.	Seropositive (%)
Stray dogs	14	2	4	5	11	78.57
Pet dogs	26	2	3	4	9	34.61
Total	40	4	7	9	20	50.00

Many workers from different parts of the world have recorded the prevalence of anti-toxoplasma antibodies by using different serological techniques i.e., Ogunrinade (1978) recorded 58% in 40 dogs from Ibadan, Southern Nigeria. The findings are in agreement with that of present study. However, lower seroprevalence of toxoplasmosis (35%) was recorded by Silva et al. (1997) in 40 dogs. The variation in infection rate may be due to different environmental and managemental conditions in various geographic areas, because toxoplasmosis is more prevalent in warm moist areas of the world than in cold or hot dry areas, a feature conducive to the survival of oocysts, shed by the cat family.

The results confirmed that the rate of seropositivity among the canines was significantly higher (78.51%) almost, double in stray dogs regardless of their age and sex as compared to indoor or pet dogs (34.61%). This may be due to the fact that the stray dogs eat dead birds, and rodents etc. and contract the infection. On the contrary, the pets avail ideal hygienic conditions and well balanced food.

As for as the age-titers relationship is concerned, the puppies below one year of age were more susceptible (62.50%) than adults (33.33%) Table 2, and that they quickly react to any slight exposure to the organisms. The similar results were recorded by Frenkel (1973). The present study further support the observations of Ogunrinade (1978) who found 52.6% of dogs below one year to be seropostive.

Table 2: Distribution of anti-toxoplasma antibodies in dogs in relation to their age and sex by using LAT.

Age/Sex Groups	No. Tested	No. of dogs Antibody titers (reciprocal) 16	128	256	Sero-positive No.	Seropositive (%)
Age						
7 months and less	25	2	6	5	13	52.00
7 month-1 year	8	1	1	3	5	62.50
2 year-3 year	4	0	0	1	1	25.00
4 year and above	3	1	0	0	1	33.33
Total	40	4	7	9	20	50.00
Sex						
Females	19	2	4	5	11	57.89
Males	21	2	3	4	9	42.85
Total	40	4	7	9	20	50.00

The seropositivity rate in females was 57.89% (11 out of 19) compared to that in males (42.85%), Table 2. Therefore, no significant difference was observed between the proportion of seropositive male and female dogs. This agrees with the findings of Ogunrinade (1978) in dogs. Aganga *et al.* (1981) also observed that the epidemiology of the disease does not appear to put either of the sex at a disadvantage as far as acquisition of infection is concerned.

Considering the breed titer relationship, the local breeds were 57.14% seropositive, whereas exotic breeds were found to be 46.15% seropositive (Table 3).

Table 3: Seroprevalence of *Toxoplasma gondii* in dogs in relation to their breed and association with cats by using LAT.

Breeds	No Tested	No. of dogs Antibody titers (reciprocal) 16	128	256	Sero-Positive No.	Seropositive (%)
Local	14	1	3	4	8	57.14
Exotic	26	3	4	5	12	46.15
Total	40	4	7	9	20	50.00
Association with cat						
Contact	14	2	2	3	7	50.00
No contact	12	0	1	1	2	16.16
Total	26	2	3	4	9	34.61

So, unlike Ogunrinade (1978) the present study revealed no significant difference in the incidence of reactors between breeds of dogs.

The seropositivity rate was found to be 50% in the dogs which have close association with cats. On contrary, it decreased significantly to 16.16% in the dogs, where owners do not let their dogs to have contact with cats (Table 3). This may be due to the reason that dogs living in association with cats eat food contaminated with oocysts of *T. gondii*, shed by cats.

From the 15 aborted bitches, the 66.66% were found to be seropostive, majority (n=7) of which gave an anti-toxoplasma antibody titer of 1:16 which indicated residual or non specific immunity. Whereas 25% seropositivity was found in the cases of normal parturition (Table 4). The study revealed a significant difference between the proportion of seropositivity in the cases of abortion and normal parturition in bitches. The results are similar to that of Okoh *et al.* (1981).

Table 4: Relationship between anti-toxoplasma antibodies and the mode of delivery in bitches by using LAT.

Mode of Delivery	No. Tested	No. of bitches Antibody titers (reciprocal) 16	128	256	Sero-positive No	Seropositive (%)
Abortion	15	7	1	2	10	66.66
Normal parturition	4	1	0	0	1	25.00
Total	19	8	1	2	11	57.89

It is epilogued that the seroprevalence of *T. gondii* in dogs is inversely related to age and that the sex and breed difference of seropositivity is negligible. However, the significant percentage seropositivity was found in roamer dogs and those having close contact with cat.

Seroprevalence of *T. gondii* in Felines

Testing of serum and faeces of apparently healthy cats for toxoplasmosis is no longer routinely recommended to assess the risk of human infection. Unless, concurrent, rigorous preventive measures are taken to reduce the possibility of infection in cats, both serologically positive and negative cats could pose a health threat to mankind.

In the current study, the overall seroprevalence of *T. gondii* in cats was recorded as 60% (Table 5).

Table 5. Prevalence of anti-toxoplasma antibodies in stray and indoor cats by using LAT.

Cat Habit	No. Tested	No. of cats Antibody titers (reciprocal) 16	128	256	Sero-Positive No.	Seropositive (%)
Stray cats	3	0	1	1	2	66.66
Indoor cats	7	2	0	2	4	57.14
Total	10	2	1	3	6	60.00

A lot of work has been done on the seroprevalence of *T. gondii* in cats in different parts of the world viz. Schomacker (1995) recorded 74.2% seropositive rate of *T. gondii* in 340 cats, Ladiges *et al.* (1982) recorded 0-94 % exposure rates of cats throughout the World. Dubey (1986) recorded 30-80% in cats in USA, Lappin *et al.* (1992) recorded 83% (59 and of 71) exposure rate in cats in USA and Chong *et al.* (1993) noted 30.3% seroprevalence of *T. gondii* in cats in Singapore. This slight variation in results could be due to different environmental and managemental conditions as well as the small sample size (n=10) in the current study. However, findings closely agree with that of Dubey (1973) in USA (59.6%).

The results have confirmed that stray cats had tendency to have higher seroprevalence (66.66%) of *T. gondii* than cats (57.14%) kept Indoor (Table 5). This may be due to the reason that stray cats could have picked up the infection through preying wild rodents, birds, reptiles, raw food scraps, etc. as reported by Dubey (1991). Mostly the cats showed antibody titer of 1:256 which was suggestive of recent exposure, followed by 1:16, indicating residual or non-specific immunity.

As for as the age titer relationship is concerned, the number of cats positive to *T. gondii* antibodies was maximum from 4 years to onward (Table 6). Similar results were recorded by Chong *et al.* (1993).

Table 6: Distribution of anti-toxoplasma antibodies in cats in relation to their age and sex by using LAT.

Age Groups	No. Tested	No. of cats Antibody titers (reciprocal)			Sero-Positive	Seropo-sitive
		16	128	256	No.	(%)
Age						
6 month and less	3	0	1	0	1	33.33
7 month-1 year	2	1	0	0	1	50.00
2 year-3 year	3	0	0	2	2	66.00
4 year and above	2	1	0	1	2	100.00
Total	10	2	1	3	6	60.00
Sex						
Females	5	1	1	2	4	80.00
Males	5	1	0	1	2	40.00
Total	10	2	1	3	6	60.00

Of the cats screened, 4 (80%) female and 2 (40.0%) male had *T.gondii* antibodies (Table 6). The seropositivity rate of *T. gondii* in exotic breeds was found to be 50% whereas that in local was 66.66% (Table 7). This difference in seropositivity is due to the fact that local breeds have more access to outside than exotic.

In nutshell, the stray cats were more prone to infection than pets and that the percentage seropositivity increased with age and there was tremendous variation in exposure rate between the males and females. The immune cats following re-exposure can shed enormous oocysts that pose a serious threat to its owners, compared to a non immune cat, which will shed only fewer organisms. So a cat giving apparently healthy look may be a seropositive, it mean the felines are not important from serologic point of view, but they are important shedder of *T. gondii* oocysts.

Table 7: Prevalence of anti-toxoplasma antibodies in different breeds of cat by using LAT.

Breeds	No. Tested	No. of cats Antibody titers (reciprocal)			Sero-positive	Seropositive
		16	128	256	No.	(%)
Persian	2	1	0	0	1	50.00
English	2	1	0	0	1	50.00
Siamese	3	0	0	2	2	66.66
Local	3	0	1	1	2	66.66
Total	10	2	1	3	6	60.00

This numerous number of seropositive dogs and cats suggests that more detailed epidemiological studies would be worthwhile in this locality.

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