

Evaluation of *Toxoplasma Gondii* IgG Antibodies in Stray and Household Dogs by Elisa

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Abstract: *Toxoplasma gondii* is one of the most prevalent multisystemic protozoal organisms affecting warm-blooded vertebrates. The aim of study was to determine *Toxoplasma* sero-epidemiology and also to investigate chronic form of the Toxoplasmosis. Blood samples were taken from 90 dogs including 45 household (pet) and 45 stray dogs in order to detect anti-*Toxoplasma* IgG antibodies. ELISA were used for diagnosing IgG antibodies. Based on our results 40% of household dogs and 77.77% of stray dogs had anti-*Toxoplasma* IgG antibodies. It was also seen that antibody titer in stray dogs was statistically much higher than that of household dogs ($P<0.05$). Antibodies titer in pets which were kept outdoor was significantly higher than that of those which were kept indoor dogs ($P<0.05$). Regarding the kind of food (raw, cooked or both) and the amount of antibody, it was seen that the antibody titer in dogs which were fed with raw food was significantly higher ($P<0.05$). Considering the sero-positivity of stray dogs it can be concluded that the way of keeping, feeding, hygiene and environmental factors have a fundamental role in animals' contact with causal agent and considering a rather high sero-prevalence in household dogs, hygienic proceedings especially using cooked food or commercially processed foods.

Key words: *Toxoplasma Gondii* · Dog · Elisa

INTRODUCTION

Toxoplasma gondii is a worldwide obligatory intracellular coccidian parasite which can infect all warm-blooded vertebrates and also human being [1, 2]. Therefore, this parasite is considered as one of the most common parasitic infections in warm-blooded vertebrates [3, 4]. Domestic cats and other felidae are definitive hosts and others are considered as intermediate hosts [5]. This parasite is transmitted to people in different ways including cats' feces, eating undercooked or raw infected meat, materials contaminated by cat's feces, blood transfusion, organ transplantation, also in congenital period from placenta [6]. Dogs usually act as mechanical vectors of the parasite and unlike cats do not show specific clinical signs [7].

The first well-documented research about *Toxoplasma* sero-positivity of Iranian dogs was conducted by Dr. Ghorbani *et al.*, 1983, by using Latex Agglutination test (LAT) method in which they were observed that 56% of stray dogs had sero-positivity [8]. The lowest sero-positivity rate of Iranian dogs is reported to be 22.4% [9], while various serologic studies which have been done in different countries indicated that the percentage of dogs sero-positivity ranges between 20% - 91% [10]. The highest rate of sero-positivity of the people in Iran (63.9%) is reported in Babol [11] and the lowest infection rate (11.5%) is in Bushehr [12], which has a high percentage in comparison with other countries such as Italy [13] and South Korea [14].

Therefore, considering the high rate of infection by *Toxoplasma* in both animals and peoples in Iran, measuring the infection rate (or sero-positivity) in

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household and stray dogs which are in a close contact with people, can prevent the mechanical transmission of parasite. This study is a high importance since few well-founded studies have been done to investigate the dogs infection in Iran; hence the present research can be considered as the first study that has simultaneously compared the rate of infection and sero-positivity in household and stray dogs and has investigated the infection and contact with this parasite in both groups of dogs (from the viewpoint of having IgM and IgG *Toxoplasma* parasite antibodies).

Controlling *Toxoplasma* is a vital issue because it is a zoonotic disease and can cause congenital problems in human infant such as abortion, neurological and eye disorders and may cause other problems in adults who are immunosuppressed [15, 16]. And also due to close contact with companion animals, there is a probability of transmission of causal agent to human specially children and pregnant women; so the investigation through screening and sero-epidemiology can be beneficial.

The aim of this study is to determine *Toxoplasma* sero-epidemiology in both household and stray dogs as well as rates of natural occurrence of infection in stray dogs and also to investigate the rates of acute form occurrence and chronic infection.

MATERIALS AND METHODS

Sampling: In order to calculate the sample size according to obtained data from the sero-positivity of dogs in Iran which is reported with an average of 39.2% (the highest rate 56% and the lowest rate 22.4%), the reliability of 95% and standard deviation of 10% were calculated.

The samples were randomly taken from available veins (especially Cephalic vein) of 45 stray and 45 household dogs in the period between January and April of 2012.

The random sampling was also done from those apparently healthy household dogs which were referred to different pet clinics in East Azerbaijan province of Iran for vaccination and periodic examinations. Some questions were asked from the owners and the information about the dogs' age, sex, mating, keeping place (house, garden or factory), keeping indoor or outdoor and nutrition type (raw, cooked or both) were recorded in the evaluation forms of each subject. Sampling from stray dogs was simultaneously done through trapping or from the dogs trapped by municipality employees and some of their apparent characteristics such as age (using dental formula) and sex were also recorded in the evaluation forms.

Table 1: Interpretation table for IgG antibodies

Result	Status
$S/P \leq 40\%$	Negative
$40\% < S/P < 50\%$	Doubtful
$50\% \leq S/P < 200\%$	Positive
$S/P \geq 200\%$	Strong Positive

Serological Assay: After blood samples' clotting in room temperature, they were immediately centrifuged 5 min at 3000×g and were used for doing evaluations after serum separation.

In order to detect anti-*Toxoplasma* IgG antibody in the samples, ID Screen *Toxoplasmosis* Indirect® (ID-VET Company, France), ELISA test were used. After keeping the frozen serums in laboratory temperature for 15-20 minutes for defrosting, the test procedure was started according to the manual provided by the producer company. For this test, at first, 90 µl of Dilution Buffer 2 was added to each microwell, then 10 µl of the Negative Control was added to wells A1 and B1 and 10 µl of the Positive Control was added to wells C1 and D1 and 10 µl of each sample to be tested was added to the remaining wells. Then, microwells were incubated for 45 min ± 4 min at the room temperature (21°C, after washing with the Wash Solution for 3 times. Then 100 µl Dilution Conjugate 1x was added to each microwell and after re-incubating them for 30 min ± 3 min at the room temperature, each well was washed again for 3 times with the Wash Solution. In the next stage, 100 µl of the Substrate Solution was added to each microwell and then was incubated for 15 min ± 2 min in the dark. After taking microwells out, 100µl of the Stop Solution was added to each well and finally they were read at 450 nm by ELISA reader. In order to interpret, the obtained percentages were read according to the interpretation table (Table 1) suggested by the producer company and the research results became specified. Eventually, the results were analyzed using T-Test, ANOVA and Chi-Square statistical methods with SPSS 17.

RESULTS

The obtained results indicated that 58.88% of dogs from (53 of 90 dogs) had anti-*Toxoplasma gondii* IgG antibody. In other words, 35 stray dogs (77.77%) and 18 household dogs (40%) had the mentioned antibody. It was also observed that antibody titer in stray dogs was significantly more than that of household dogs ($P < 0.05$). Although there was no significant difference between IgG antibodies titer and the stray dogs sex (male 234.93 and

female 217.20, $P>0.05$), in case of household dogs, antibody average in male dogs was statistically more significant than that of female ones ($P>0.05$).

Although in both groups of dogs (stray and household) antibodies titer increased with age, but there was no significant correlation between age and antibodies ($P>0.05$). Present study indicated that there is no significant difference between dogs' breeds and dogs sero-positivity ($P>0.05$). The results also indicated that the antibodies titer in the stray dogs was significantly more than that of household ones ($P<0.05$). In the present study, the household dogs were also compared in terms of keeping indoor and outdoor and it was observed that the antibodies titer in those kept outdoor was significantly more than that of dogs which kept indoor ($P<0.05$).

The relationship between the kind of food and antibody amount was also investigated and it was observed that the dogs which were fed only with cooked food had the lowest and those which were fed with raw food had the highest antibody amount and antibody average in dogs which were fed only with raw food was significantly higher than that of dogs which were only fed with cooked food ($P<0.05$).

DISCUSSION AND CONCLUSION

Toxoplasmosis is one of the most prevalent parasitic diseases of the world, because it is able to infect most of the warm-blooded vertebrates [4]. Despite complete recognition of the parasite's life cycle, unfortunately there is a high occurrence of infection, especially in Third World countries as well as developed ones [17, 18]. Hence, sero-epidemiologic investigation and approving essential precautions to preventing infection of people and companion animals are of great significance. Despite considering cats as the only hosts with the ability of oocyst shedding to the environment [19, 20], the infection can be transferred to human by the dogs mechanically by direct excretion of oocyst [7].

In this study it was observed that 58.85% of dogs, i.e. 40% of household and 77% of stray dogs in East Azerbaijan province had anti-Toxoplasma IgG antibodies. Considering positive serum occurrence in 20 to 91% of dogs in the world [10] the obtained results of this study indicate a rather high occurrence of sero-positivity serum against this parasite; and although infection rate is somewhat higher in the region, the existence of Toxoplasma antibodies is not notably high compared to

other parts of the world [18]. Anti-Toxoplasma antibody titer amounts have also been measured in other countries [17, 21-23]; so it can be concluded that Toxoplasma antibody titer of dogs in Iran, is similar to other countries. The first well-founded research in Iran was done in 1983 and it was observed that 56% of stray dogs in Iran had anti-Toxoplasma antibody [8] while in other researches 29.7% [24] and 22.4% [9] of dogs had positive serum antibodies. Unfortunately, no other researches have been done for investigating dogs' sero-positivity in Iran except for the mentioned ones. Also no studies have been done to compare household and stray dogs to investigate acute form of the disease and serum infection simultaneously.

Comparing IgG antibody titer levels in stray and household dogs, it can be clarified that the antibody titer average in stray dogs is significantly higher than the household ones ($P<0.05$). Also it is obvious that stray dogs, because of living in nature, with low hygienic conditions, eating raw food or sometimes infected animals' carcasses and vector animals, have a higher contact with the causal agent than the household dogs. Although the results of this study are consistent with those of other studies [25], high antibody amounts in the animals without clinical signs and low antibody amounts in infected animals have also been observed [3, 17]. So it seems that the kind of animals has a direct relationship with the contact with causal agent and the amount of antibodies titer levels.

In various studies it has been proved that the antibody amount increases with age of the animal, which increases the possibility of its contact with parasite [5, 26]. But in this research although the antibody amount has increased with the age increasing of both stray and household dogs, the antibody titers increase in both groups is not statistically significant ($P>0.05$). This can be as a result of investigating antibody amounts in young dogs, since the age average of dogs in this research has been around 36 months.

Based on our results, no significant difference was observed between the dogs' sex and IgG antibody titer in stray dogs' group ($P>0.05$); but in household dogs' group the antibody titer in males was significantly more than that of females ($P<0.05$). Although in some similar studies, there has been a relative and sometimes significant difference between sex and antibody rate in cats [1, 27] according to the knowledge of author no significant relationship between dogs' antibody rate and their sex is reported. To the author, this difference can be as a result of high number of male dogs ($n=27$) in comparison with

female ones (n=18) and since male dogs are mostly used for guarding in factories, villas and other outdoor places, the possibility of their contact with parasite increases and this can lead to significant difference of antibody in these dogs.

The results also indicated that dogs which were kept in gardens and unprotected places, had the most and those which were kept in houses or protected places, had the least serum antibodies titer and antibody titer average in the first group was significantly more than that of the second group ($P<0.05$). This can occur according to keeping situation of the dogs because the environmental conditions, nutrition and keeping these dogs is completely different from the household ones and therefore, the possibility of their contact with this parasite (or contact with infected water and food or vector animals) is high; so, because these dogs are usually kept outdoor and can be infected through contact with other animals or by hunting some of them such as rodents and vector insects, there has been a significant difference and as a result they show a high amount of antibody. Furthermore, most of these dogs eat raw food and this can justify the difference between antibody averages in them and in household dogs. In the present study those dogs which were kept in house also were compared in terms of keeping indoor and outdoor and it was observed that antibody average in dogs which were kept outdoor was significantly more than that of those which were kept indoor ($P<0.05$). This emphasizes the significant role of hygiene, keeping environment and nutrition in decreasing the infection rate by the parasite. Similar to other studies, also a significant relationship in case of food type was seen and according to the obtained results it was observed that those animals which used raw food had higher serum antibodies in comparison with those which were fed with cooked food ($P>0.05$); and this indicated how hygiene and keeping conditions can have a significant role in increasing serum antibodies.

The results also showed that because of the existence of tissue cysts and high prevalence of them in mammals' muscles, after ingestion, these cysts can infect animals and as a result increase antibody rate [5]. This is also true about human since those people who use raw or undercooked food had high serum antibodies [16]. According to most of the references, meat infection has a fundamental role in contact with the parasite and getting infected [4], so this research also supports the results of other researchers' studies. However, recently some researchers consider other infection ways such as ingestion of oocyst, existing in water which can cause the

disease. In a research which has been conducted by Jonese et al. in 2010, it was observed that being infected with *Toxoplasma* can be occurred through ingestion of oocyst existing in infected materials (such as water) in addition to ingestion of tissue cysts [28].

Toxoplasma disease diagnosis using clinical signs is very difficult; and although sampling from some tissues such as neural tissue can be used for *Toxoplasmosis* diagnosis [29, 30], using this method requires advanced equipments and high precision and it is also expensive. Moreover to conduct these studies, many animals are killed for sampling, so using sero-epidemiology method for diagnosing acute and chronic cases is of a high priority rather than the mentioned methods. Meanwhile, *Toxoplasma* disease is frequently occurred subclinical conditions [2] and in case of being suspicious to most of the diseases such as Neosporosis, Canine Distemper, Systemic Mycosis and etc. differential diagnosis should be done [2]. Because of this, nowadays in similar studies, serologic methods which have proper characteristics and sensitivity are used for diagnosing *Toxoplasma* in dogs and other animals [20, 31-34].

It has been observed that simultaneous measurement of IgG and IgM antibodies in a serum sample, offers more reliable results about existence or non-existence of infection and also about elapsed time after infection in canine and feline [5]. Therefore, considering using ELISA method for measuring serum antibodies and also immuno-chromatography method in acute form of the disease simultaneously, it seems that the results of this study have had the ability to separate the occurrence of contact with the parasite and acute infections and also have investigated disease occurrence and sero-prevalence both in household animals and those kept in outdoor places. The results obtained from this study indicated that unfortunately serum antibody against *Toxoplasma* parasite is high in stray animals but existence of a proper amount of serum antibody in household animals exactly shows the role of nutrition and hygiene in household animals. Hence, in order to prevent infection in companion animals, educating animal owners, feeding animals with cooked food and preventing the entry of vector animals and insects to environment, are essential and of great significance. Fortunately, considering dogs as a mechanical transmitter of infection to human, preventing companion dogs' contact with infected cats, preventing them from eating infected cats' feces and treating feces-eating habit in companion dogs, have a vital role to decrease the infection rates in dog owners.

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