

RESEARCH ARTICLE



Toxoplasma gondii Infection in Cats from Southwest Areas of Albania

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Abstract

Cats are the natural reservoir of *Toxoplasma gondii* and excrete the resistant oocyst to environments. The Mediterranean climate is favourable for the stable development of many arthropod species, which are incriminated as vectors for various agents. In the present study, the presence of *T. gondii* antibodies was examined in 138 domestic household referred cats to private animal clinics in Gjirokastra and Tepelena, from October 2012 to January 2014 by modified agglutination test (MAT). *T. gondii* antibodies were detected in 58 (42 %) cats. The antibody titers in the positive animals ranged from 1:25 to 1:500. Our results show *T. gondii* is widespread in domestic cats in southwest of Albania. The highest prevalence (48.2 % of 41) was in outdoor cats. The prevalence of *T. gondii* antibodies in 42% of cats suggests wide-spread contamination of the rural environment with oocysts.

Keywords: Seroprevalence, risk, cats, *Toxoplasma gondii*, health.

1. Introduction

Toxoplasmosis caused by *Toxoplasma gondii*, is an endemic parasitic zoonosis that occur throughout the world. It infects humans as well as a broad spectrum of vertebrate hosts. The definitive host for *T. gondii* is cat and the intermediate hosts are mammals and birds. People can get infected by ingestion of tissue cysts with raw or undercooked meat, and by ingestion of oocysts with water, vegetables or soil [20]. It has been suggested that *T. gondii* infection with oocysts is more serious than infection with tissue cysts [21]. The most common clinical form of human toxoplasmosis is lymphadenitis but the major clinical problem of toxoplasmosis is congenital infection of fetus resulted from primary infection during pregnancy, as well as ocular toxoplasmosis and reactivated form in immunocompromised patients [27]. Cats play an important role in the spread of toxoplasmosis because they are the only animals that excrete resistant oocysts into the environment [34].

Stray cats and dogs in the Albania are becoming a public concern because there is a considerable increase in their number annually. These facts indicate the risk of mechanically spreading zoonoses including toxoplasmosis to humans since human acquire the infection from infected mammals, either directly or

indirectly. Many epidemiological studies on *T. gondii* in cats have been published. Usually the presence of antibodies rather than oocyst shedding is detected. The standard diagnosis of toxoplasmosis in cats is based on coprological diagnosis. However, the results are difficult to interpret since oocysts of *T. gondii* in cat feces are usually few in number [8]. Serological surveys for the detection of anti-*T. gondii* antibodies in cats was used to assess the degree of environmental contamination as the antibodies persist and indicate prior exposure to *T. gondii* [31]. Different assays are in use for the detection of antibodies against *T. gondii* in cats, but test evaluation is often lacking even though this is a prerequisite before drawing any conclusions from the results obtained with the test [23]. A seroprevalence survey of Toxoplasma in Tirana showed that 91 cats (62.3%) of stray and household cats were positive for Toxoplasma antibody [33]. Antibodies to *T. gondii* (titre 1:100) were demonstrated in this study. There has been no national estimate of *T. gondii* prevalence in cats in the Albania. Serologic testing of clinically ill cats with toxoplasmosis as a differential diagnosis resulted in an overall seroprevalence of 31.6% [36]. The present study was designed to determine the seroprevalence of *T.gondii* infection in domestic and stray cats in Tepelena and Gjirokastra areas (southwest of Albania), by using the MAT.

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2. Material and Methods

2.1. Study population

Serum samples were obtained from cats in some private clinics while the cats were under general anesthesia for surgery. The cats were presented to the private clinics in different rural and urban areas of Tepelena and Gjirokastra. The cat populations were divided into indoor cats and outside or in-out cats. The barn cats were those that lived in barns and were handled by the owners of the farms. The outside or in-out cats were those that were handled, lived inside the house or outside, and were up-to-date in their vaccinations.

2.2. Serological assay

A total of 138 cats (53 household and 85 outside or in-out cats) of various ages and of both sexes were referred to private animal clinics of Tepelena and Gjirokastra, from October 2012 to January 2014 for various ailments. Their blood

samples were collected from Jugular veins. These blood samples were left for about an hour for blood clotting to occur. The clotted blood was then separated with a fine loop immediately and was centrifuged at 3500 rpm for 10 minutes. The separated sera were stored at -20°C until assayed. Sera were diluted 1:25, 1:50, and 1: 500 with phosphate buffer saline and tested by the modified agglutination test (MAT), as described by Dubey and Desmonts (1987). On the basis of extensive evaluation in cats fed tissue cysts, a titer of 1:25 was considered indicative of *T. gondii* infection in cats [9, 12, 13, 14].

3. Results and Discussion

The overall seroprevalence of *Toxoplasma gondii* in cats was 42 percent. Antibodies to *T. gondii* were found in 58 out of 138 (42%) cats: 6 at titers of 1:25, 13 at 1:50, and 39 at 1:500. Thus, 67 % of seropositive cats had high (1:500) antibody titers (Table 1). Sero-positive cats were found in all areas examined (Table 1).

Table 1. Prevalence of *T. gondii* antibody and the MAT titers in cats in study areas

Sex	No. Examined	No. (%) Positive	No. of cats with antibody in titers		
			1:25*	1:50*	1:500*
Male	57	23 (40.4)	2 (3.5)	6 (10.5)	18 (31.6)
Female	81	35 (43.2)	4 (5.0)	7 (8.6)	21 (25.9)
Total (%)	138	58 (42.0)	6 (4.4)	13 (9.4)	39 (28.3)

*Serum dilution

Antibodies to *T. gondii* were found in 23 out of 57 (40.4 %) males and 35 out of 81 (43.2 %) females. The proportion of females testing positive for *T. gondii* antibodies was not significantly higher than that of males ($p>0.05$). The prevalence of *T. gondii* infection was highest in cats >3 years old

(63.4%) (Table 2). The seropositivity rate of *Toxoplasma gondii* in young and adult cats showed that there is a relationship between seropositivity and age ($p>0.05$).

Table 2. Seroprevalence of *Toxoplasma gondii* antibodies in cats according to age

Age of cats	Number of cats studied	No. (%) Positive	No. of cats with antibody in titers		
			1:25*	1:50*	1:500*
Juvenile, >6-12 months	44	7 (15.9)	0	2 (4.6)	5 (11.4)
Young adult, >12-36 months	53	25 (47.2)	2 (3.8)	5 (9.4)	18 (33.9)
Adult, >36 months	41	26 (63.4)	4 (9.8)	6 (14.6)	16 (39.0)
Total (%)	138	58 (42.0)	6 (4.4)	13 (9.4)	39 (28.3)

* Serum dilution

The seropositivity rate of *Toxoplasma gondii* increased with age. The outside cats were more prone to infection than indoors (Table 3).

Table 3. Seroprevalence of *Toxoplasma gondii* antibodies in cats according to habitat

Habitat	No. Examined	No. (%) Positive	No. of cats with antibody in titers		
			1:25*	1:50*	1:500*
Household	53	17 (32.1)	1 (1.9)	5 (9.4)	11 (20.8)
Outside	85	41 (48.2)	5 (5.9)	8 (9.4)	28 (32.9)
Total (%)	138	58 (42.0)	6 (4.4)	13 (9.4)	39 (28.3)

Seroprevalence of *T. gondii* in cats varies depending on the type of cats (feral vs. domestic), age of cats, method of serologic testing, and geographic location. Household and stray cats often live freely out and in human houses. In addition to their natural food, cats feed on garbage discarded around the houses in night, this is important because they discharge some helminthic eggs and protozoan cysts into the environment, transmittable to human. Food source of these animals is important in the transmission and for the completion off the life cycle and cats are definitive host, play a pivotal role in the epidemiology of toxoplasmosis [18]. Due to close contact of cats with human and this fact that children play outdoors on the soil, cats can be an important potential source of transmission of zoonotic parasite such as *Toxoplasma* because they are the only hosts that can excrete the resistant *T. gondii* oocysts into the environment [18]. Based on its zoonotic nature, toxoplasmosis is one major public health issue worldwide and thus monitored closely in human medicine, but it is also considered as an important cause of reproductive disease in small ruminants. The MAT method was chosen for this study, because of its high specificity and sensitivity, as well as its simple application and usage with no cross-reactivity with other infective organism of cat [10], also this test is used to get comparative serological data on naturally infected cats [17, 32]. Diagnosis of *T. gondii* infection in cats is based on detection of oocysts in faeces by microscopy, bioassay or PCR or detection of antibodies by serology. Nevertheless, definitive diagnosis of toxoplasmosis in cats can be difficult to accomplish. First of all, because microscopy is not sensitive, the limit of detection is 1000 oocysts/g faeces (Dubey, personal communication), and *T. gondii* oocysts cannot be differentiated microscopically from *H. hammondi* oocysts. On the other hand, bioassay is time consuming, expensive, relies on the presence of sporulated (infective) oocysts and requires animal facilities. Moreover, cats excrete oocysts for a short period of about 2 weeks, mainly once in their life when a primary infection takes place

[11]. IgG antibodies are initially absent during primary infection, but after some weeks rise to protective levels and remain detectable for years. IgM antibodies rise within days, and usually decrease over a few weeks. However, in some cats with chronic infection IgM persists [15]. The results of this study have confirmed that outside or in-out cats 41 (48.2 %) had tendency to have higher seroprevalence than cats kept indoor 17 (32.1 %). This may be due to the reason that outside or in-out cats could have licked up the infection through catching of wild rodents, birds, reptiles, raw food scraps etc as reported by Dubey (2004). In the previous study, of 146 domestic cats surveyed in Tirana during 2008 through 2010, antibodies to *T. gondii* were found in 91 cats (62.3%), and antibodies were measured by the indirect fluorescent antibody test (IFAT) [33]. In this study the prevalence of *T. gondii* infection in referred cats to private veterinary clinics in Tepelena and Gjirokastra areas in southwest of Albania is 42 %. Also world-wide toxoplasma prevalence ranges in cats from 5.4% to 90 % [30, 25]. However, the prevalence of IgG antibody of *T. gondii* reported in this study is comparable to other studies from Albania and other regions of the world. The Mediterranean Basin is considered as a region where zoonoses are widespread and most numerous as regards variety, but often receives insufficient attention to specifically assess its situation. The data of this study indicate a high endemicity of *T. gondii* in the southwest of Albania because all seropositive cats had probably already shed oocysts and contaminated the environment; cats can shed millions of oocysts after ingesting few bradyzoites [16]. Because oocysts are rarely found in feces of cats, serologic prevalence data in cats are important for the determination of epidemiologic significance of *T. gondii* infection [12]. At any given time only about 1% of cats were found shedding *T. gondii* oocysts [8]. Toxoplasmosis is widespread in south-east Europe [3]. The average prevalence of IgG antibodies was reported to be approximately 50% in pregnant woman in Albania [28] and among Albanians who migrated to Italy [35] and this percentage of exposure represents the upper limit of

the range of currently reported data from the Balkans [2, 5, 29]. Recently seroprevalences of 47% to 81% were reported in cats in Romania [4, 24] or of almost 48% in Hungary [26]. Consistent with several other studies, the *T. gondii* seroprevalence in the cats from Tirana and southwest of Albania was positively associated with the age of the cats [24, 26]. In our study, the overall seroprevalence of *Toxoplasma gondii* in cats 42 percent was lower than that of stray cats 91 (62.3 %) and adult cats 26 (83.9 %) reported by Silaghi et al. (2014) in Tirana. This variation is probably related to differences in the timing of the studies, the environmental conditions responsible for the dissemination of *T. gondii* infection [8], season of sampling and differences of sensitivities and specificities of used tests and the distribution of samples. The high prevalence of *T. gondii* antibodies in the cats from Tirana, Albania, is certainly related to their origin from suburban habitats with constant access to the outdoors, which has been identified as a risk factor of the infection [24]. Most cats in Albania are raised either outdoors or both outdoors and indoors. Since infected stray cats shed oocysts around public places, healthy animals and humans may get infected due to the contaminated environment. This study also reinforces the role of stray cats as one of the potential sources of toxoplasmosis transmission to humans in Albania. The chance of contracting toxoplasma through ingestion of oocysts is very high as the percentage of cats in the recent infective stage is very high. Keeping in view these findings, it is recommended that pets should be confined inside to prevent hunting and should be fed only dry canned or cooked food. Litter box should be changed daily, preferably not by pregnant women. Used pet litter should not be disposed off in yard or garden. Human should eat only well cooked meat products. Hands should be washed thoroughly after handling meat, vegetables and cats and also after gardening. Beside consumption of undercooked meat, 'direct soil contact' has been thought to be associated with the seropositivity [28]. In most surveys, the sex of dogs was not significantly associated with seroprevalence, as has been reported by other researchers [1, 6]. On the other hand, the rate of seropositive female cats was significantly higher than that of seropositive male cats. In Japan, no significant differences were observed in the seroprevalence of *T. gondii* in both the sexes of cats [30]. The gender-based differences in

seroprevalence between stray dogs and cats in Albania are presently unclear.

4. Conclusions

In conclusion, we suggest that stray cats have an important role in contamination of environment to oocysts but it is not clear whether they have been infective by ingestion of oocysts or by eating the meat of intermediate host especially small rodent and birds. Some studies on such intermediated host are necessary in this region. This information is important for public health, because cats are one of the most popular pets in Albania and frequently come into close contact with humans [22]. These cats may contaminate the environment, thus exposing humans, and particularly children, to possible *T. gondii* infection.

5. References

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