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■ BRIEF COMMUNICATION

Prevalence of *Toxoplasma gondii* in Stray Cats of Gyeonggi-do, Korea

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Abstract: *Toxoplasma gondii* is an obligate intracellular zoonotic protozoan with a worldwide distribution. It infects humans as well as a broad spectrum of vertebrate hosts. Cats and wild felidae play crucial roles in the epidemiology of toxoplasmosis. This study was performed to survey the prevalence of *T. gondii* infection among stray cats in the Gyeonggi-do, Republic of Korea. A total of 174 stray cat blood samples were collected from Gwacheon-si (n = 20), Bucheon-si (82), and Yangju-si (72). Positive sera for *T. gondii* were identified in 14 samples (8.1%) exclusively via the latex agglutination test, 28 (16.1%) via ELISA, and 23 (13.2%) via PCR analysis. The overall infection rate of female stray cats (29.2%) presented as higher than that of male cats (24.0%). This study suggests that *T. gondii* is widespread in the stray cat population of Gyeonggi-do, Korea. It is urgently needed to control urban stray cat population and to reduce the risk of zoonotic transmission of toxoplasmosis to other animal hosts and humans.

Key words: *Toxoplasma gondii*, stray cat, latex agglutination test, ELISA, PCR

Toxoplasma gondii is a worldwide parasite that can infect the central nervous system of warm-blooded animals, including humans. The infection is acquired mainly by eating food or water contaminated with oocyst or tissue cysts of *T. gondii* [1]. A zoonotic infection of this parasite leads to an asymptomatic infection in healthy persons. Clinical toxoplasmosis appears to occur by reactivation of the infection in individuals who are immunocompromised, especially patients with acquired immunodeficiency syndrome (AIDS) or cancer [2]. Cats play an important role in the spread of toxoplasmosis because they are the only animals that excrete resistant oocysts into the environment [3]. In the Republic of Korea, large numbers of stray cats are found roaming residential streets and increasing the risk of public health for animals and humans. The present study was performed to determine the prevalence of *T. gondii* in a Korean stray cat population, as this frequency is important for determining the epidemiological significance of *T. gondii* infection.

A total of 174 stray cats (75 males and 99 females) were assayed for the prevalence of *T. gondii* using the latex agglutination test (LAT), ELISA, and diagnostic polymerase chain reaction (PCR). Twenty samples were collected from Gwacheon-si, 82 from Bucheon-si, and 72 from Yangju-si in Gyeonggi-do, Korea. Stray cats were captured as the TNR (trap, neuter, return) program, promoted by the government from April to October 2007. Blood was collected from each cat by cephalic or jugular venipuncture, allowed to clot, and centrifuged for 5 min at 1,800 g, and then the serum was collected and stored at -20°C until used. The presence of *T. gondii* antibodies was analyzed using a LAT kit (Eiken Chemical Co., Tokyo, Japan). The procedures described in the manufacturer's instructions were followed accurately. Briefly, serial 2-fold dilutions of the sera were prepared to 1 : 2,048 titer. After mixing the sensitive latex suspension for at least 12 hr, titers measuring 32 or higher were recorded as positive. ELISA was performed as previously described in Choi et al. [4]. The cutoff absorbance of 0.25 was recorded as a positive reaction. For diagnostic PCR, 2 pairs of oligonucleotide primers directed against the B1 gene of *T. gondii* were used to perform nested PCR [5], using a Maxime PCR premix Kit (Intron, Korea). PCR reactions were cycled 40 times with denaturation at 93°C for 10 sec followed by annealing at 57°C for 10 sec and finally an extension step at 72°C for 30 sec. The amplified DNA was visualized following separation in 2% agarose gels.

In LAT, *T. gondii* antibodies were detected in 14 (8.1%) of 174

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Table 1. Number and percentage (%) of stray cats seropositive for *Toxoplasma gondii* captured at different cities

City (Number of samples; n)	LAT			ELISA			PCR		
	Female	Male	N* (%)	Female	Male	N* (%)	Female	Male	N* (%)
Bucheon (82)	3	2	5 (6.1)	11	4	15 (18.3)	8	9	17 (20.7)
Gwacheon (20)	3	-	3 (6.7)	3	-	3 (6.7)	1	1	2 (10.0)
Yangju (72)	4	2	6 (8.3)	6	4	10 (13.9)	2	2	4 (5.5)
Total (174)	10	4	14 (8.1)	20	8	28 (16.1)	11	12	23 (13.2)

*Values indicate the number of seropositive cats by each test.

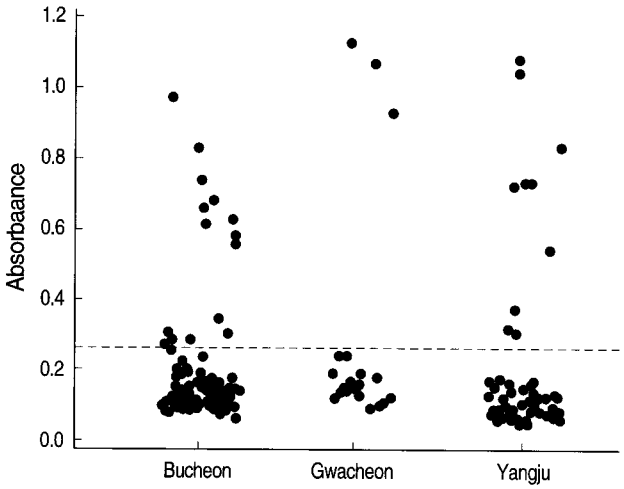

Fig. 1. *Toxoplasma gondii* IgG antibody titers in sera of stray cats caught in each area by ELISA.

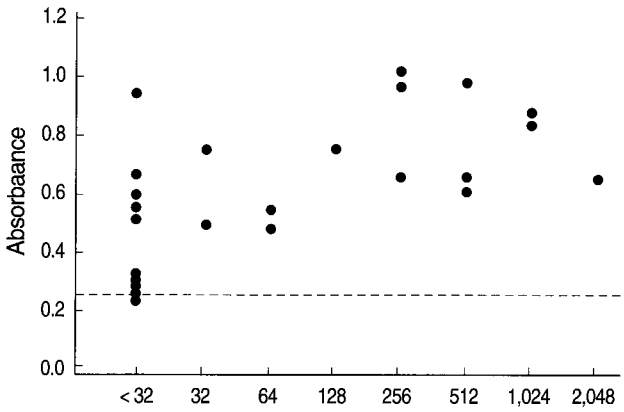

Fig. 2. *Toxoplasma gondii* IgG antibody levels in sera of stray cats by ELISA to serial LAT dilution titers.

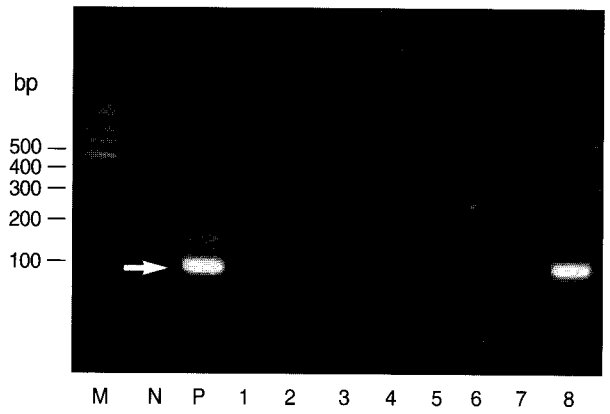

Fig. 3. Amplification of 96 bp B1 PCR product from stray cat blood DNA in 2% agarose. M, 100 bp ladder; N, negative control; P, positive control (genomic DNA from *T. gondii*); 1-8: Blood DNA extracted from Gwacheon-si stray cats.

cat sera. The seroprevalence of *T. gondii* according to districts is shown in Table 1. A total of 14 sera (4 males and 10 females) were positive and their positive rates for each region were as follows: 5 (6.1%) of 82 Bucheon-si samples, 3 (6.7%) of 20 Gwacheon-si samples and 6 (8.3%) of 72 Yangju-si samples were positive for *T. gondii*. Sera of the stray cats were tested for specific IgG antibodies to *T. gondii* via ELISA, which indicated 28 (16.1%) positive out of 174. In Bucheon-si, 15 (18.3%) of 82 sera were positive, and 3 (6.7%) of 20 sera in Gwacheon-si and 10 (13.9%) of 72 sera in Yangju-si were positive (Figs. 1, 2). Among the 28 positive sera, 8 were male and 20 were female. Following analysis of the *T. gondii* B1 gene via diagnostic PCR, 23 (12 males and 11 females; 13.2%) of 174 sera exhibited positive reactions (Fig. 3). In Bucheon-si, 17 (20.7%) of 82 sera showed positive reactions. In Gwacheon-si and Yangju-si, 2 (10.0%) of 20 sera and 4 (5.5%) of 72 sera were positive, respectively. There was no significant difference between gender, regions, and test ($P > 0.05$).

A lot of tests have been used to find the positive infection with *T. gondii* such as LAT, ELISA, indirect hemagglutination assay (IHA) and PCR for various target genes. The LAT and ELISA are now widely available for serological diagnosis of toxoplasmosis. Serological surveys are good indicators of the occurrence of *T. gondii* infection in cats because serologically positive cats probably shed oocysts [6]. The development of a highly sensitive and specific PCR protocol to identify *T. gondii* DNA will help in the early diagnosis of toxoplasmosis [5]. In the present study, the

positive rate of *T. gondii* infection was 8.1% with LAT, 16.1% with ELISA, and 13.2% with PCR analysis. ELISA showed a higher positive rate than other tests. Therefore, ELISA is a more effective screening test for *T. gondii* infection than LAT and PCR.

Free-living animals such as stray cats, boars, and foxes could be surveyed as indicators of environmental spreading of *T. gondii*. Stray cats are especially important in Korea because they occupy the highest position of the urban food chain and have increased their numbers gradually [7]. In previous reports on the prevalence of *T. gondii* in Korea, 37.0% (17/46) of cats raised on Jeju island were seropositive by ELISA [8], 13.1% (26/198) of stray cats in a rural area near Chinju-si were positive by sandwich-ELISA [9], and 20.7% of 212 stray cats from 5 regions were positive by indirect immunofluorescent antibody assay [9]. The seroprevalence of *T. gondii* in cats has been shown to vary depending on their type (stray or domestic), age, method of testing, and geographic location [10]. The present study also represented different prevalences of *T. gondii* in each region of Korea. Especially, Han et al. [11] reported that the seropositive rate in Seoul and Gyeonggi-do was 31.9%, though the sampling area in Gyeonggi-do was not documented. The positive rate of *T. gondii* in the present study was significantly lower ($P < 0.05$) than that reported by Han et al. [11]. This result may be related to the density of animals in each district and environmental conditions in Gyeonggi-do.

Our current study represents that *T. gondii* is widespread in stray cat populations of Gyeonggi-do. Further studies in various areas will be necessary to survey the overall epidemiological status of toxoplasmosis of stray cat populations in Korea. Control programs of urban stray cat populations are needed in order to reduce the risk of zoonotic transmission of toxoplasmosis to animals and humans.

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