Korean J Parasitol. Vol. 48, No. 3: 267-270, September 2010 DOI: 10.3347/kjp.2010.48.3.267

▣ BRIEF COMMUNICATION

Prevalence of *Toxoplasma gondii* Infection in Stray and Household Cats in Regions of Seoul, Korea

Sang-Eun Lee[1,†], Jae-Yeong Kim[2,†], Yun-Ah Kim[1], Shin-Hyeong Cho[1], Hye-Jin Ahn[3], Heung-Myong Woo[2], Won-Ja Lee[1] and Ho-Woo Nam[3,*]

[1]Division of Malaria and Parasitic Diseases, National Institute of Health, Korea Centers for Disease Control and Prevention, Seoul 122-701, Korea; [2]Laboratory of Surgical Medicine, Veterinary Medical College, Kangwon National University, Chuncheon, Korea; [3]Department of Parasitology and Catholic Institute of Parasitic Diseases, Catholic University, Seoul 137-701, Korea

Abstract: The principal objective of this study was to investigate the prevalence of toxoplasmosis in household and stray cats in Seoul, Republic of Korea. We collected blood samples from 72 stray and 80 household cats, and all samples were examined by ELISA and nested PCR. The overall positive rates of *Toxoplasma gondii* in stray cats were 38.9% (28/72), with 15.3% (11/72) in ELISA and 30.6% (22/72) in PCR. The positive rate in male stray cats was slightly higher than that of female stray cats. The highest positive rate of *T. gondii* infection was noted in Gangnam and Songpa populations in ELISA and in Gwangjin population in PCR. In household cats, however, we could not detect any specific antibodies or DNA for *T. gondii*. In conclusion, we recognized that the infection rate of toxoplasmosis in stray cats in Seoul was considerably high but household cats were free from infection.

Key words: *Toxoplasma gondii*, cat, ELISA, PCR

Toxoplasma gondii is a worldwide endemic parasite, and a member of the phylum Apicomplexa, subclass coccidian. It can infect most warm-blooded animals and humans, and can occasionally cause permanent neurological damage, with or without hydrocephalus, or chorioretinitis. Infections with this zoonotic agent are acquired principally by eating food or water contaminated with oocysts or tissue cysts of *T. gondii*. Cats have an important role in the spread of toxoplasmosis, because they are the only animal that excretes resistant oocysts into the environment. A single cat may shed more than 100 million oocysts into the environment [1,2]. Internationally, the presence of large populations of feral cats have become a controversial issue, due to their impact on cat overpopulation, animal welfare, public health, and the environment, and also due to disagreements about the best methods for their control [3]. Also in Korea, large numbers of stray cats are found roaming residential streets and have become a target of public grievances. They disrupt people's sleep, make noise, and cause traffic accidents, and this has been a fairly serious issue since 2000. In addition, they threaten the population as a source of zoonotic diseases, including toxoplasmosis. Chai et al. [4] successfully isolated and maintained the *T. gondii* parasite from a Korean ocular patient, which was designated the Korean Isolate-1 (KI-1).

Seroprevalence of *T. gondii* infection in stray and household cats were reported in Spain [5], Tehran [6], China [7], and Japan [8]. Whereas in Korea, several epidemiological surveys of *T. gondii* in just stray cats have been conducted in recent years [9-11], and no researchers have, until now, conducted a comparison of prevalence of *T. gondii* infections in household and stray cats in the same area. It should be noted that the precise disposition of the household cat population remains obscure. Therefore, the present study was first conducted to investigate the prevalence of *T. gondii* infection in stray and household cats in Seoul, Korea, via ELISA and nested PCR analyses.

A total of 80 household cats and 72 stray cats were subjected in this study. Blood samples of household cats were collected whenever they were admitted to a veterinary clinic by their owners for regular health check-ups. On the other hand, blood samples of stray cats were received from other veterinary clinics, located in the same areas where the household cats' samples were obtained, through the local government's TNR (Trap, Neuter, Return) program, the purpose of which was to reduce the numbers and control the natural reproduction rates of urban stray cats. A total 9 areas, Jungrang, Nowon, Dongdaemun, Gang-

• Received 14 April 2010, revised 28 June 2010, accepted 30 June 2010.

* Corresponding author (howoo@catholic.ac.kr)

† Both authors equally contributed to this work.

nam, Gwangjin, Seongbuk, Dobong, Songpa, and Gangbuk, in Seoul were surveyed (Fig. 1). All samples were collected from April to September 2008. Blood samples were collected from cephalic or jugular punctures of each cat, permitted to clot at room temperature and centrifuged for 5 min at 1,800 *g*. Serum and whole blood were collected after centrifuging and transferred to the Division of Malaria and Parasitic Diseases at the Korea Centers for Disease Control and Prevention.

General methods of DNA extraction were carried out by the manufacturer's instructions using the DNeasy Mini column kit (Qiagen, Hilden, Germany). The DNA concentration of each blood sample was measured with a Quant-iT™, dsDNA HS Assay Kit (Invitrogen, Carlsbad, California, USA) and read by Qubit™, Fluorometer (Invitrogen), and the average DNA concentration was 15 ng and the range was a minimum 7 ng to a maximum 46 ng. All samples were stored at -20℃ until used. ELISA for toxoplasmosis was performed essentially in accordance with the procedure of Choi et al. [12] and a nested PCR targeting the specific B1 gene for *T. gondii* was followed according to the condition with Kim et al. [9]. The PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, California, USA) and sequenced by a commercial laboratory (Macrogen, Seoul, Korea).

Our study involved 72 stray cats (32 males and 40 females) and 80 household cats (40 males and 40 females, spay 4, and aged 4 months to 7 years) and they had no specific symptoms in external appearance. As the seroprevalence of *T. gondii* in cats varied depending on their living type (stray or domestic), age, the diagnostic method used, and geographic area [13], the prevalence of *T. gondii* in stray cats has been reported to be variable from 5% to 45% in recent years in Korea [9-11].

The results of this study were similar to the previous reports. The overall positive population of *T. gondii* in stray cats was 38.9% (28/72); the rate was 15.3% (11/72) by ELISA and 30.6% (22/72) by nested PCR (Table 1). The positive rate of *T. gondii* in nested PCR analysis was 2 times higher than that of ELISA and the positive samples only in ELISA showed absorbance of 0.4-0.9 (over the 0.25 cut-off value), while the range of posi-

Table 1. Comparison of ELISA and PCR analysis for detecting *Toxoplasma gondii* infection in stray cats

		ELISA		Total
		Positive	Negative	
PCR	Positive	5	17	22
	Negative	6	44	50
	Total	11	61	72

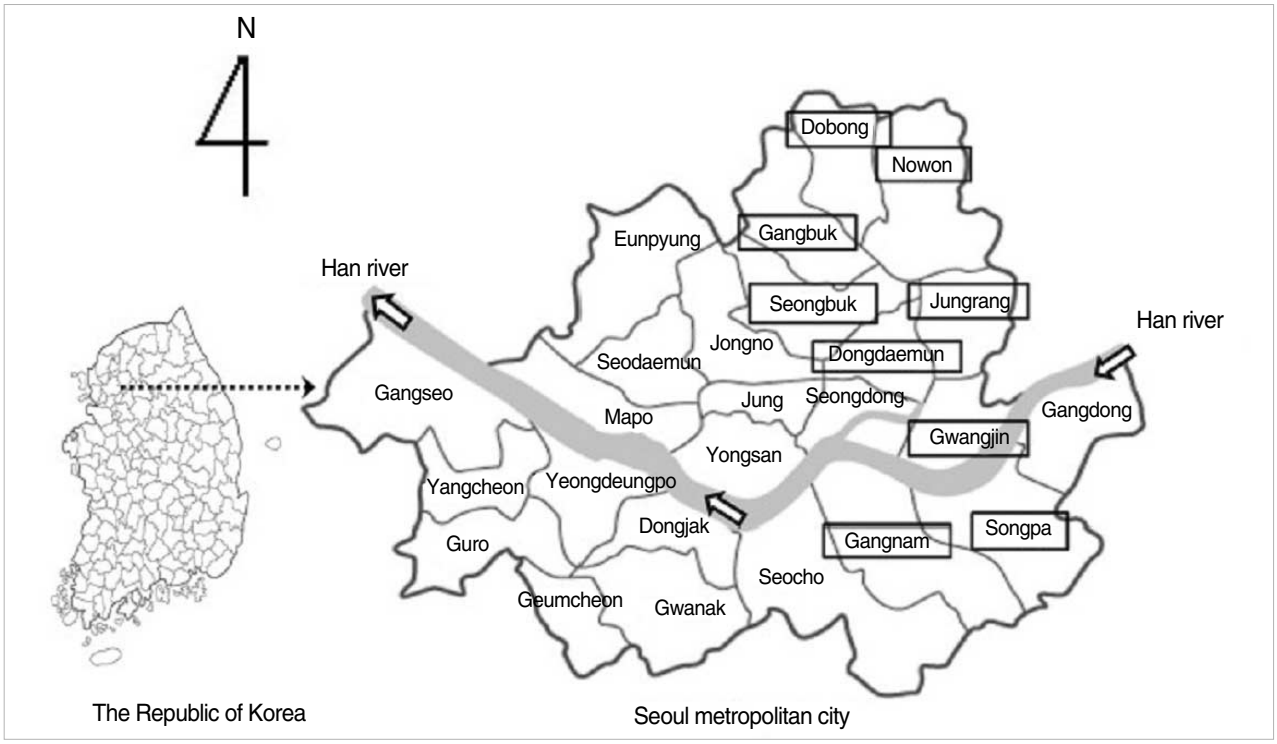

Fig. 1. Areas surveyed (rectangles) for *Toxoplasma gondii* infection in stray and household cats in Seoul, Korea.

Table 2. Result of *Toxoplasma gondii* infection with ELISA and PCR analysis in stray and household cats in each area of Seoul

		Jungrang		Nowon		Dongdaemun		Gangnam		Gwangjin		Sungbuk		Dobong		Songpa		Gangbuk		Total	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Stray cats	Number	1	3	3	2	10	7	3	3	11	21	-	-	-	-	3	3	1	1	32	40
	ELISA	-	1	-	1	2	2	2	-	1	-	-	-	-	-	1	1	-	-	6	5
	PCR	-	-	-	1	2	1	1	1	5	10	-	-	-	-	1	-	-	-	9	13
Household cats	Number	9	11	8	4	5	2	8	7	1	4	2	4	2	2	2	1	3	1	40	40

M, male; F, female.

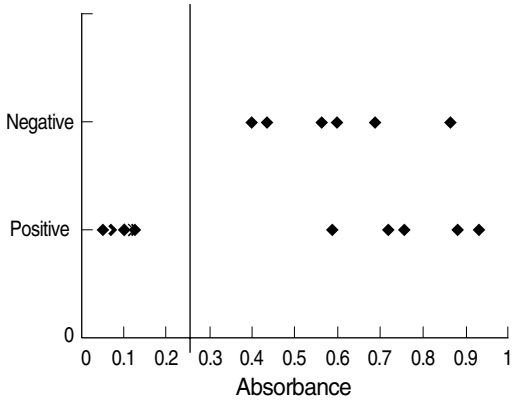

Fig. 2. ELISA evaluation for nested PCR positive and negative samples.

tives in both ELISA and nested PCR was wider, from 0.1-0.9 (Fig. 2). This was similar to the reports by Kim et al. [9] and the development of a highly sensitive and specific PCR will help in the early diagnosis of toxoplamsosis in blood [14,15]. In terms of sex, the male positive rate was 18.8% (6/32) and the female rate was 12.5% (5/40) in stray cats by ELISA, and 28.1% (9/32) in males and 32.5% (13/40) in females by nested PCR (Table 2). The positive rate of *T. gondii* in male cats was slightly higher than that in females. Similar [5] or opposing [6,7,9] results have been reported. Regionally, the prevalence of *T. gondii* varied and the highest positive rates of *T. gondii* infection were noted in Gangnam and Songpa for ELISA, and Gwangjin for nested PCR (Table 2). However, it was not possible to directly compare the prevalences of *T. gondii* infection in different areas because of a difference in the sample numbers and environmental characteristics of each region.

Our study of household cats was relatively important, because the prevalence of *T. gondii* in household cats in Korea has never been assessed until now. According to reports in other nations, the prevalence of *T. gondii* in household cats was 25.5% in Spain [5], 36% in Tehran [6], 17.9% in China [7], and 5.4% in Japan [8]. In most reports, the prevalence of stray cats was higher than that of household cats in the same area. However, interestingly, we could not detect any specific antibodies and DNA of *T. gondii* in household cats. By this interesting result, the authors could assume that most household cats are managed quite well by their owner's. Stray cats in Seoul are important wild animals because they occupy the highest position in the urban food-chain and their numbers gradually increased [10]. These cats establish territory in the same fashion as wild animals and generally kept out of other cats' way in each area. Therefore, it is assumed that the life cycle of *T. gondii* is maintained in the territory of each cat and in each area. Concurrently, a potential zoonotic threat to humans and animals remains a concern in all of them [9].

In conclusion, toxoplasmosis is widespread among stray cats of Seoul, whereas household cats are not infected. Further epidemiologic surveys of toxoplasmosis in stray cats from various areas in Seoul and studies of the life styles of household cats will be need in order to gain a more complete understanding of *T. gondii* infection.

ACKNOWLEDGEMENTS

This work was supported by a grant (NIH-4847-302-210-13, 2009) from the National Institute of Health, Korea Centers for Disease Control and Prevention.

REFERENCES

1. Frenkel JK, Biology of *Toxoplasma gondii*. In Ambroise-Thomas P, Peterse E eds, Congenital toxoplasmosis: scientific background, clinical management and control. Paris, France. Springer-Verlag. 2000, p 9-25.
2. Omata Y, Oikawa H, Kanda M, Mikazuki K, Nakabayashi T, Suzuki N. Experimental feline toxoplasmosis: humoral immune responses of cats inoculated orally with *Toxoplasma gondii* cysts and oocysts. J Vet Med Sci 1990; 52: 865-867.
3. Wallace JL, Levy JK. Population characteristics of feral cats admit-

ted to seven trap-neuter-return programs in the United States. J Feline Med Surg 2006; 8: 279-284.
4. Chai JY, Lin A, Shin EH, Oh MD, Han ET, Nam HW, Lee SH. Laboratory passage and characterization of an isolate of *Toxoplasma gondii* from an ocular patient in Korea. Korean J Parasitol 2003; 41: 147-154.
5. Miro G, Montoya A, Jimenez S, Frisuelos C, Mateo M, Fuentes I. Prevalence of antibodies to *Toxoplasma gondii* and intestinal parasites in stray, farm and household cats in Spain. Vet Parasitol 2004; 126: 249-255.
6. Haddadzadeh HR, Khazraiinia P, Aslani M, Rezaeian M, Jamshidi S, Taheri M, Bahonar A. Seroprevalence of *Toxoplasma gondii* infection in stray and household cats in Tehran. Vet Parasitol 2006; 138: 211-216.
7. Zhang H, Zhou DH, Zhou P, Lun ZR, Chen XG, Lin RQ, Yuan ZG, Zhu XQ. Seroprevalence of *Toxoplasma gondii* infection in stray and household cats in Guangzhou, China. Zoonoses Public Health 2009; 56: 502-505.
8. Maruyama S, Kabeya H, Nakao R, Tanaka S, Sakai T, Xuan X, Katsube Y, Mikami T. Seroprevalence of *Bartonella henselae*, *Toxoplasma gondii*, FIV and FeLV infections in domestic cats in Japan. Microbiol Immunol 2003; 47: 147-153.
9. Kim HY, Kim YA, Kang SW, Lee HS, Rhie HG, Ahn HJ, Nam HW, Lee SE. Prevalence of *Toxoplasma gondii* in stray cats of Gyeonggi-do, Korea. Korean J Parasitol 2008; 46: 199-201.
10. Lee JY, Lee SE, Lee EG, Song KH. Nested PCR-based detection of *Toxoplasma gondii* in German shepherd dogs and stray cats in South Korea. Res Vet Sci 2008; 85: 125-127.
11. Sohn WM, Nam HW. Western blot analysis of stray cat sera against *Toxoplasma gondii* and the diagnostic availability of monoclonal antibodies in sandwich-ELISA. Korean J Parasitol 1999; 37: 249-256.
12. Choi WY, Nam HW, Youn JH, Kim DJ, Kong Y, Kang SY, Cho SY. Detection of antibodies in serum and cerebrospinal fluid to *Toxoplasma gondii* by indirect latex agglutination test and enzyme-linked immunosorbent assay. Korean J Parasitol 1992; 30: 83-90.
13. Dubey JP, Saville WJ, Stanek JF, Reed SM. Prevalence of *Toxoplasma gondii* antibodies in domestic cats from rural Ohio. J Parasitol 2002; 88: 802-803.
14. Jones CD, Okhravi N, Adamson P, Tasker S, Lightman S. Comparison of PCR detection methods for B1, P30, and 18S rDNA genes of *T. gondii* in aqueous humor. Invest Ophthalmol Vis Sci 2000; 41: 634-644.
15. Paugam A, Dupouy-Camet J, Sumuyen MH, Romand S, Lamoril J, Derouin F. Detection of *Toxoplasma gondii* parasitemia by polymerase chain reaction in perorally infected mice. Parasite 1995; 2: 181-184.