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# Serological Observation of *Toxoplasma Gondii* Prevalence in *Apodemus Agrarius*, a Dominant Species of Field Rodents in Korea

Soung-Hoo Jeon and Tai-Soon Yong

## Abstract

Field rodents involved in ecological food chains and which are the prey of carnivores in the natural environment may serve as reservoir hosts for *Toxoplasma gondii* infection in humans, however, no data has been published to date in Korea. A total of 1,008 *Apodemus agrarius*, a dominant species of field rodents in Korea, were trapped at various locations around the country, and their serum antibody (IgG) levels to *T. gondii* were examined by ELISA. The mean absorbance was 0.11, and fifteen samples (1.49%) showed positive titers from 0.18 to 0.59. The seropositive samples were analyzed by immunoblot. Five of them showed reactive bands to *T. gondii* water soluble antigens of 30, 35, and 43 kDa. This immunoblot analysis showed very similar patterns to that obtained using sera of experimentally infected mice with *T. gondii*. The present study presents indirect evidence of the existence of *T. gondii* in field rodents in Korea.

Key Words: *Toxoplasma gondii*, *Apodemus agrarius*, prevalence, antibody

## INTRODUCTION

*Toxoplasma gondii* is an obligate intracellular parasite that causes significant morbidity and mortality in humans throughout the world. *T. gondii* also infects all kinds of mammals and some birds. The organism has been isolated from herbivorous, carnivorous and omnivorous animals.[1]

In Korea, Soh et al. reported *T. gondii* skin test positive rate in 5.6% of 373 examinees for the first time.[2] And also reported on another seroepidemiological study which indicated that *T. gondii* infection was related with neurological or physical disorders in humans.[3] Choi et al. described two cases of chorioretinitis patients, a six-year-old child with congenital toxoplasmosis and a 26-year-old female with acquired toxoplasmosis.[4] Choi et al. reported two outbreaks of acute toxoplasmosis involving 8 adult patients, who had eaten raw pork.[5]

Dubey et al. conducted a survey on swine and wild lives in 47 swine farms, to identify sources and reservoirs of *T. gondii* infection, and found that the *T. gondii* antibody positive rate was 2.1−68.3%.[1] The presence of *T. gondii* was confirmed in the brains and hearts of rodents trapped on farms, therefore, the rodents were confirmed as reservoirs of *T. gondii* infection.

Field rodents are herbivores and therefore, probably become infected by ingesting food or water contaminated with oocysts. Since the existence and distribution of *T. gondii* in field rodents in Korea have not been studied until now, the present study was performed to elucidate the seroprevalence rate of *T. gondii* in *Apodemus agrarius*, a dominant species of field rodents in Korea.

## MATERIALS AND METHODS

### Trapped sites of *Apodemus agrarius*

Thirteen regions and 49 sites in Korea were selected to capture *Apodemus agrarius* during December 1990 and November 1997 (Fig. 1, Table 1). They were trapped at the levee of a rice paddy after harvest and at ridges of ordinary fields using Sherman live traps. Each trap was baited with 20 g of oats-peanut butter ball set up with 2−3 m intervals at 5−6 P.M.

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Department of Parasitology, Yonsei University College of Medicine, Seoul, Korea.
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Address reprint request to Dr. S. H. Jeon, Department of Parasitology, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea. Tel: 82-2-361-5290, Fax: 82-2-363-8676, E-mail: shjeon1201@yahoo.co.kr

**Table 1. The Prevalence Acute of Anti-*Toxoplasma Gondii* Antibodies of *Apodemus Agrarius* Trapped between December 1990 and November 1997 at 49 sites in Korea**

| Locality | | Seropositive/Tested | Prevalence (%) rate |
|---|---|---|---|
| Seoul: | (1) Kaehwa-dong, Kangso-gu | 0/2 | 0 |
| Inchon: | (2) Oryu-dong, Kyesan-gu | 0/10 | 0 |
| | (3) Yungjong-do | 0/6 | 0 |
| Kyonggi-do: | (4) Tokchong-ri, Yangju-gun | 1/19 | 5.3 |
| | (5) Kwangtan 3-ri, Paju-gun | 0/49 | 0 |
| | (6) Pugok 1-dong, Puchon-shi | 0/7 | 0 |
| | (7) Dorae 5-ri, Koyang-gun | 3/112 | 2.7 |
| | (8) Namsan-ri, Kanghwa-gun | 0/22 | 0 |
| | (9) Kyopo-ri, Pyongtaek-shi | 0/43 | 0 |
| | (10) Sanchong-ri, Pochon-gun | 0/12 | 0 |
| Taejon: | (11) Tandong, Yosong-gu | 0/4 | 0 |
| Chungchongbuk-do: | (12) Shimok-ri, Chong-won-gun | 1/40 | 2.5 |
| | (13) Sosong 2-dong, Chungju-shi | 0/4 | 0 |
| Chungchongnam-do: | (14) Pongan-ri, Kongju-shi | 0/10 | 0 |
| | (15) Toyang-ri, Nonsan-shi | 0/13 | 0 |
| | (16) Toksan-myon, Yesan-gun | 0/1 | 0 |
| | (17) Yongpo-ri, Yon-gi-gun | 0/4 | 0 |
| | (18) Kosan-ri, Tangjin-gun | 0/20 | 0 |
| | (19) Naejang-ri, Hongsong-gun | 0/13 | 0 |
| Kang-won-do: | (20) Kundok-ri, Samchok-gun | 0/15 | 0 |
| | (21) Chongok-dong, Tonghae-shi | 3/16 | 8.8 |
| | (22) Chonchong-ri, Chunchon-shi | 0/19 | 0 |
| | (23) Munhye 1-ri, Chorwon-gun | 0/5 | 0 |
| Chollabuk-do: | (24) Haechon-ri, Wanju-gun | 0/9 | 0 |
| | (25) Shinyong-dong, Iksan-shi | 0/91 | 0 |
| | (26) Changshin-ri, Iksan-shi | 0/64 | 0 |
| | (27) Unsan-ri, Puan-gun | 0/2 | 0 |
| Chollanam-do: | (28) Migok-ri, Hwasun-gun | 0/4 | 0 |
| | (29) Kunso-myon, Yonggwang-gun | 0/10 | 0 |
| | (30) Yong-wol-ri, Hampyong-gun | 0/9 | 0 |
| | (31) Namsan-ri, Changhung-gun | 0/10 | 0 |
| | (32) Chohwa-ri, Yochon-gun | 1/21 | 4.8 |
| Kwangju: | (33) Won-dong, Kwangsan-gu | 1/11 | 9.1 |
| | (34) Uchi-dong, Puk-gu | 0/10 | 0 |
| Kyongsangbuk-do: | (35) Yopae 2-ri, Kimchon-shi | 0/19 | 0 |
| | (36) Chiksan-ri, Yechon-gun | 0/22 | 0 |
| | (37) Chinhyon-dong, Kyongju-shi | 0/7 | 0 |
| | (38) Unsu 2-ri, Kimchon-shi | 0/12 | 0 |
| | (39) Chikha-ri, Andong-shi | 0/29 | 0 |
| | (40) Cho 1-dong, Sangju-shi | 0/32 | 0 |
| | (41) Chinan-ri, Mun-gyong-shi | 0/21 | 0 |
| | (42) Pukyong-ri, Yongdok-gun | 0/19 | 0 |
| Kyongsangnam-do: | (43) Ka-po-dong, Masan-shi | 0/24 | 0 |
| | (44) Toson-ri, Tong-yong-gun | 0/15 | 0 |
| Cheju-do: | (45) Tongkwi-ri, Pukcheju-gun | 0/36 | 0 |
| | (46) Kumdok-ri, Pukcheju-gun | 1/42 | 2.4 |
| | (47) Otung-dong, Cheju-shi | 0/2 | 0 |
| | (48) Ara 1-ding, Cheju-shi | 0/31 | 0 |
| | (49) Kosong-ri, Pukcheju-gun | 4/10 | 40.0 |
| Total (%) | | 15/1,008 | 1.49 |

PAGE and then electrophoretically transferred to a nitrocellulose (NC) membrane using the method of Towbin et al.[7] Membranes were incubated with each serum sample with positive ELISA titers and sera of controls. NC strips were blocked for 1 hr with 3% BSA in 20 mM PBS, pH 7.4 containing 0.05% Tween 20. After several washes, the strips were incubated with 1 : 100 diluted primary antisera, and then with AP conjugated goat anti-mouse IgG (1 : 1000) antibody. The substrate (NBT/BCIP: Promega, WI, USA) for AP was added and incubated for 5 min at RT. The reaction was stopped by rinsing the strips in water.

## RESULTS

The results of ELISA for 1,008 serum samples of *A. agrarius* collected at 49 trapped sites are given in Table 1. The mean absorbance of 1,008 serum samples was 0.11 (±0.033). The mean absorbance of antibody titers of 22 mice infected with *T. gondii* ME49 tissue cysts at 3 weeks and 6 weeks post-infection, 22 mice immunized with *T. gondii* RH tachyzoite lysate at 6 weeks postimmunization and 22 negative control mice were 0.23 (±0.08), 0.57 (±0.15), 0.85 (±0.17) and 0.08 (±0.05), respectively (Fig. 2). Absorbances greater than the mean plus two standard deviations of the absorbance of seronegatives was considered positive. Fifteen of 1,008 serum samples were found to be antibody positive (0.18−0.59) against *T. gondii* RH strain lysate. Table 1 shows the prevalence rate of anti-*Toxoplasma* antibodies of *A.*

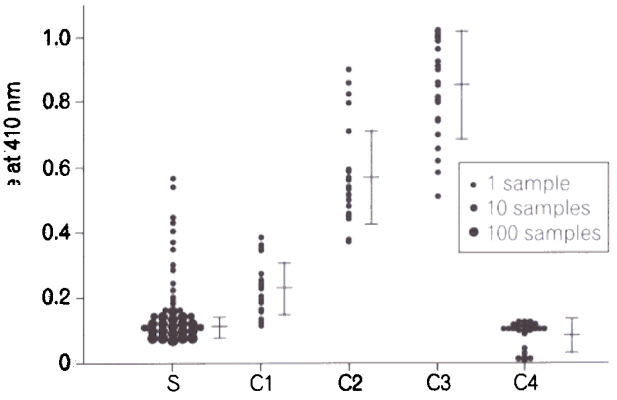


*Fig. 2. The geometric mean value (±SD) of ELISA absorbance of the 1,008 sample group (S) was 0.11 (±0.033). The mean absorbance of the antobody titers of 22 mice infected with ME49 tissue cysts at 3 weeks (C1) and 6 weeks (C2) postinfection, 22 mice immunized with RH tachyzoite lysate at 6 weeks (C3) postimmunization and 22 negative control mice (C4) were 0.23 (± 0.08), 0.57 (±0.15), 0.85 (±0.17) and 0.08 (±0.05), respectively.*

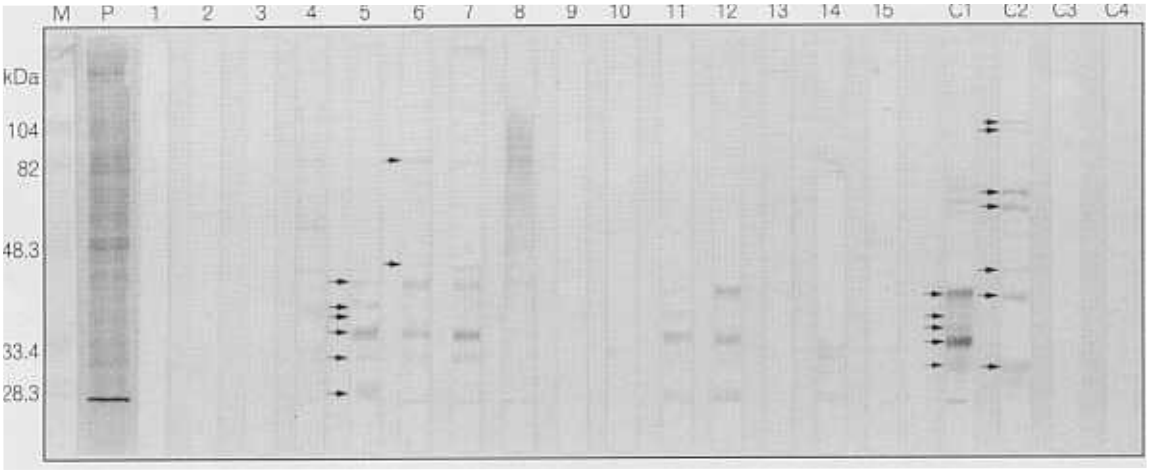


*Fig. 3. Immunoblot analysis of* A. agrarius *sera (1−15) showing reactive bands against T. gondii (RH strain) lysate, especially at 30, 34 and 41 kDa. Note a similar reactive band pattern in the lane C1 of ME49 strain of T. gondii infected. The band pattern using immune serum with T. gondii (RH strain) lysate was different in lane C2. M: Marker, P: T. gondii lysate, 1−15: Seropositive serum samples of A. agrarius, C1: Mouse serum infected with ME49 tissue cyst, C2: Mouse serum immunized with RH tachyzoite lysate, C3: Seronegative serum sample of A. agrarius, C4: Control serum of normal mouse. Arrows of 5 and 6 strips indicate 81, 43, 41, 37, 35, 34, 30 and 28 kDa from top to bottom (C1: 65, 59, 41, 37, 35, 34, 30; C2: 116, 105, 65, 59, 43, 41, 30 kDa).*

*agrarius*. Out of 49 trapped sites, seropositive animals were found at 8 sites. The overall positive rate for *T. gondii* was 1.49% (n=15/1,008). The positive rate by locality was highly variable, with the highest being found at Kosong-ri, Cheju-do (40.0%, n=4/10), and the next at Chongok-dong, Kang-won-do (18.8%, n=3/16), whereas the lowest rate was shown at Kumdok-ri, Cheju-do (2.4%, n=1/42).

Of 1,008 samples, 15 positive sera and the extract from RH and ME49 strain were analyzed by immunoblotting (Fig. 3). Of 15 positive samples, 5 showed prominent band patterns, and significantly strong IgG responses to proteins with molecular weights (MWs) of 28, 30, 34, 35, 37, 41, 43 and 81 kDa were found in five sera from Cheju-do (lanes: 5, 6, 7) and Kang-won-do (lanes: 11, 12). The negative control sera and negative *A. agrarius* sera measured by ELISA showed no bands or only a few very faint bands. The ME49 strain infected sera reacted to 30, 34, 35, 37, 41, 59 and 65 kDa proteins. The RH strain immunized sera reacted to 30, 41, 43, 59, 65, 105 and 116 kDa proteins.

## DISCUSSION

From an epidemiological point of view, studies on population densities of field rodents are very important because they are involved in the ecological food chains of mammals in natural environment and may serve as one of the reservoir hosts in the transmission of *T. gondii* to humans. In this study, *A. agrarius*, which is very widely distributed, was found to be the predominant (94.3%, n=1,008/1,068) species of field rodents, followed by *Mus musculus* (1.9%), *Crocidura laisura* (1.8%), *Microtus fortis* (1.1%), *Micromys minutus* (0.4%), *Rattus norvegicus* (0.2%), *Eotheromys rufocanus regulus* (0.1%), *Mustela siberica* (0.1%) and *Rattus rattus* (0.1%). Nineteen *Crocidura laisura,* insectivores, were captured. Twenty *Mus musculus,* two *Rattus norvegicus* and one *Rattus rattus,* which were originally domestic rodents, were collected in the field. *A. agrarius,* a predominant species of field rodent in Korea could serve as an important reservoir host as subordinate of ecological niche of mammals for *T. gondii* transmission to human.In particular, high Anti-*Toxoplasma* IgG level samples were found primarily in animals captured near farmhouses. So herbivores, such as, *A. agrarius* seemed to have been in infected by ingesting farm products or water contaminated with the oocysts in cats' faeces.

A total of 216 animals in captivity at the Seoul Grand Park in Korea were examined by Choi et al., based on antibody titers to *T. gondii* by indirect latex agglutination test.[8] Twenty out of 131 mammals (15.3%) and 2 out of 75 birds (2.7%) showed positive antibody titers, and none among rodents (Indian giant squirrel). With the exception of the exercises mentioned in this paper, the existence and distribution of *T. gondii* have not been investigated to date in the Korean environment. In this study, the authors carried out an epidemiological study on *T. gondii* in nature.

ELISA is one of the most useful serological methods, because a large number of sera can be examined in a relatively short time.[4] Although, the cut-off value in ELISA was determined as mean+2SD of negative controls in this study, 5 (0.54), 6 (0.44), 7 (0.59), 11 (0.32), and 12 (0.45) absorbance values of the sera of strips with prominent bands on immunoblotting were greater than the mean+3SD (0.224).

From 1981 to 1990, the prevalence of *T. gondii* infection in small mammals was investigated at various locations in the Czech Republic, and the prevalence rate was reported as 7.4% (n=7/94) in *A. agrarius*.[9] As compared with the results of our study, the prevalence rate in *A. agrarius* in the Czech Republic was much higher. The difference in the seroprevalence rate of *T. gondii* infection in rodents between the Czech Republic and Korea may reflect an actual difference in its prevalence. The difference also might have been caused by differences between the trapping locations or reasons.

Immunoblot analysis showed reactive bands to *T. gondii* water-soluble antigens from 28 to 81 kDa in 5 samples sera (Fig. 3). In particular, the reactive bands at 30, 34, 35, 37 and 41 kDa were from field mouse sera, which were also recognized by the sera of ME49 strain infection. The reactive band of 43 kDa was also recognized in the sera of RH strain immunization. The best characterized of these are the three MWs of 30, 35 and 43 kDa associated with the surface antigens of similar size, described by Couvreur et al.[10] Kasper et al.[11] and Handman et al.[12] The MW of 41 kDa seemed similar to the immunoblot of the 5 sample sera and mouse antisera to ME49 and RH strain. This molecule, the stage-specific protein of tachyzoite, which was associated with

internal organelles, has previously been reported.[13] Tomavo et al. have described 4 bradyzoite surface molecules with MWs of 18, 21, 34 and 36 kDa. The present study also showed reactive bands to proteins with MWs of 34 and 37 kDa, although it is not clear whether these proteins are corresponding bradyzoite surface antigens.[14] The 28 kDa molecule reacts only weakly, and may be identical to cytoplasmic molecules of similar size, as described by Weiss et al.[15] On the other hand, there were no bands, or only few very faint bands with sera of seronegative samples or the serum of the negative control.

The present study presents indirect evidence of the existence of *T. gondii* in field rodents in Korea. Further studies are required to provide definite evidence for the existence of *T. gondii* in field rodents, for an example, by the detection of *T. gondii* by nested polymerase chain reaction technique using species-specific primers or the primary isolation of the parasite.

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