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# Prevalence of serum antibodies to *Toxoplasma gondii* in the small Indian mongoose (*Herpestes auropunctatus*) on Amami-Oshima Island, Japan

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**ABSTRACT.** Prevalence of antibodies to *Toxoplasm gondii* was studied using the latex agglutination (LA) method, followed by sucrose density gradient centrifugation (SDGC) method on the small Indian mongoose (*Herpestes auropunctatus*), which inhabits Amami-Oshima Island. Of the 362 samples, 38 (10.5%) revealed positive. Single or double peaks in the 7–8 and/or 12–14 fraction to LA titer by SDGC indicated the early stage of *T. gondii* infection. It is suggested that domestic/feral cats play an important role for spreading this zoonotic pathogen to the mongoose as well as other species that are endemic to this island. Future studies are warranted to prevent the transmission of *T. gondii* among cats and wild animals in order to maintain the ecosystem health.

**KEY WORDS:** Amami-Oshima Island, small Indian mongoose, *Toxoplasm gondii*

The small Indian mongoose (*Herpestes auropunctatus*) is a carnivorous animal that belongs to the family Herpestidae and is listed among the worst 100 invasive alien species worldwide [11]. This species inhabits two islands of Japan, namely Okinawa Main Island and Amami-Oshima Island, and their presence extensively impacts the islands' endemic species [7, 22]. A national conservation program to eliminate this species has been considered under the Invasive Alien Species Act [13]. The population of the small Indian mongoose on the Amami-Oshima Island, a large island (712.35 km$^2$) located between Kyushu and Okinawa Islands of Japan (28°27′33″ North, 129°36′34″ East) was estimated as 10,000 in 2000; whereas in 2018, due to the extensive measures taken for eliminating this species, the population drastically reduced to 50 [17].

Although several studies have reported some pathogens infecting the small Indian mongoose present Okinawa Main Island [6, 10], no pathogen-related studies are available for Amami-Oshima Island. *Toxoplasma gondii*, a zoonotic protozoan parasite, which infects felines as the final host as well as various vertebrates including human and birds as intermediate hosts [5]. Little is known about *T. gondii* infection in mongoose world-wide [1, 3, 4, 20]. This study aimed to determine the incidence of *T. gondii* infection in the small Indian mongooses that inhabits Amami-Oshima Island.

Serum samples from 362 mongooses (188 adult males, 128 adult females, 23 juvenile males, 22 juvenile females, and 1 sex/age unknown) were supplied by the Amami Wildlife Conservation Center collected and stored frozen at −20°C during the period from 1989 to 2005. Each animal was categorized as a juvenile or adult according to its age based on the eruption of milk and permanent teeth. Individuals with remaining milk teeth and erupting permanent teeth were all considered as juveniles.

The Toxocheck$^{TM}$ antibody test (Eiken Chemical Co., Ltd., Tokyo, Japan), was used for *T. gondii*, and the antibody titer was measured by latex agglutination (LA) method. According to the instructions of the kit, humans, pigs and cats usually gives a positive reaction when the antibody titer is 16 times or more [14, 15]. In this study, the positive values were placed 32 times or more using Smirnov Grubbs test ($P$<0.05) in statistics [18], since measurement by experimental infection could not be performed in the species. The specimens with an antibody titer of or higher than 64 times were subjected to immunoglobulin fractionation using sucrose density gradient centrifugation in order to estimate infection time [2, 14, 16], wherein the test serum was diluted

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**Table 1.** Antibody titers to*Toxoplasma gondii* among the small Indian mongoose (*Herpestes auropunctatus*) by age class and sex in Amami-Oshima Island

| Age & Sex | Titer of antibody | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | <2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | |
| Juvenile male | 18 | 1 | 2 | 0 | 1 | 1 | 0 | 0 | 23 |
| Juvenile female | 6 | 0 | 14 | 0 | 1 | 1 | 0 | 0 | 22 |
| Adult male | 34 | 17 | 111 | 9 | 9 | 7 | 0 | 1 | 188 |
| Adult female | 25 | 2 | 80 | 4 | 5 | 9 | 1 | 2 | 128 |
| Unknown[a)] | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Total | 84 | 20 | 207 | 13 | 16 | 18 | 1 | 3 | 362 |

a) No information of samples about age class and sex.

by 5-fold with a phosphate buffer, placed on a 10–40% sucrose density gradient and was ultra-centrifuged (35,000 rpm) at 4°C. *T. gondii* antibody titer was measured by the LA method for the 23 fractions obtained by the aforementioned method. Difference between males and females was analyzed by student's *t* test and a value of $P \leq 0.05$ was considered as statistically significant.

Of 362 samples analyzed, 38 (10.5%) (17 adult males, 17 adult females, 2 juvenile males and 2 juvenile females) were positive for *T. gondii* infection (Table 1). Despite various methods used for antibody testing, the prevalence rate in this study is lower than that of the same species inhabiting Grenada of West Indies. Moreover, the Indian gray mongoose (*H. edwardsii*) inhabits Saudi Arabia and the Egyptian mongoose (*H. ichneumon*) inhabits Spain (30%, 67%, and 59%, respectively) [1, 4, 20]. The antibody titer measurement in the immunoglobulin fraction using sucrose density gradient centrifugation revealed a single peak between fractions 11 and16 in 18 samples (6 adult males, 11 adult females, 1 juvenile male), a single peak between fractions 7 and 8 in 3 samples (2 adult males, 1 adult female), and two peaks between fractions 7–8 and 12–14 in 1 sample (1 adult female). A single peak found in the fraction 7–8 presumably indicates a reaction to IgM and that in fraction 11–16 indicates IgG [16]. Since no reports are available on the immunoglobulin changes after *T. gondii* infection in the small Indian mongoose, it is difficult to estimate the time of infection. However, IgM and IgG generally appear in the acute phase within 2–4 weeks and after 3–6 weeks post experimental inoculation, respectively [9]. It is reported that domestic cats (*Felis silvestris catus*) lose IgM after 16 weeks after post infection [8], and so it is supposed a response to IgM of the 3 small Indian mongooses is the infection within 16 weeks or less on Amami-Oshima Island.

*T. gondii* isolates from species inhabiting Grenada of West Indies were belonged to the Type III (ToxoDB #2), ToxoDB #7, and ToxoDB #216 lineages [4]. Meanwhile, Cheng *et al.* (2018) found negative heart-PCR result from small Indian mongoose [3]. The aforementioned serological and genotyping data indicating the role of mongoose in the life cycle and epidemiology of *T. gondii*. Further studies are needed to clarify the prevalence and genotypes of *T. gondii* circulating among domestic/feral cats on Amami-Oshima Island.

Mongoose generally feeds on small mammals such as rodents, birds, amphibians and reptiles. *T. gondii* infection found in this alien species indicates the predation of infected mice or ingestion of oocysts excreted in feces of domestic/feral cats [12]. The population of feral cats is increasing on Amami-Oshima Island, and they act as predators for certain endangered endemic species such as the Amami rabbit (*Pentalagus furnessi*), the Ryukyu long-tailed giant rat (*Diplothrix legata*) and the Amami spiny rat (*Tokudaia osimensis*) which are all Natural monument of Japan [19]. Notably, an Amami spiny rat died due to *T. gondii* infection by ingesting oocysts from cats [21]. Presumably, *T. gondii* can be transmitted among domestic/feral cats, the small Indian mongoose, and some endemic mammals on the island. Although the history of introduction of cats into Amami-Oshima Island is not well known, *T. gondii* infection found in wild small Indian mongooses indicates that domestic/feral cats spread this zoonotic pathogen on the island where wild cat species do not exist [12].

As mongoose is dead-end host for *T. gondii* in this area, it could be used as an indicator of environmental contamination. *T. gondii* infection among domestic/feral cats needs to be studied for risk assessment for endemic species and to plan control measures against the transmission of this zoonotic pathogen to the wild animals on the island, thereby maintaining the ecological balance.

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