SEROLOGIC DETECTION OF *TOXOPLASMA GONDII* INFECTION IN *RATTUS* SPP COLLECTED FROM THREE DIFFERENT SITES IN DASMARIÑAS, CAVITE, PHILIPPINES

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Abstract. Acute and chronic cases of toxoplasmosis in *Rattus norvegicus* and *Rattus rattus mindanensis* caught in agricultural, commercial and residential sites in Dasmariñas, Cavite, Philippines were determined serologically. Fifty-eight percent of *R. norvegicus* and 42.0% of *R. r. mindanensis* were positive for anti-*T. gondii* antibodies (Abs). Infection was higher in male rats, and those caught in the commercial site had 100.0% seropositivity. Thirty percent of the *R. norvegicus* and 51.0% *R. rattus mindanensis* had acute infection, with 1:64-1:128 Abs titer. Seventy percent of the *R. norvegicus* and 49.0% of *R. rattus mindanensis* were chronically-infected with Abs titer 1:256-1:2048 and 1:256-1024, respectively. The association between the presence of infection with the rat gender and species and their collection sites was insignificant ($p>0.05$). In a related study, however, mice experimentally-inoculated brain tissue homogenate obtained from chronically-infected *Rattus* spp, manifested differences in the onset as well as, severity of infection which was histopathologically evaluated, suggestive of a possible difference in *T. gondii* parasite strain(s) infecting different rat populations.

INTRODUCTION

Rattus species are the most diverse among the rodents, of which 20 species are considered important pests (Fall, 1980). In the Philippines, the two most important pestiferous rats that survive and proliferate around human habitation include *Rattus norvegicus* and *Rattus rattus mindanensis* (Fernando *et al*, 1985). Besides the agricultural and domestic damages caused by rats, they are also carriers of human disease, including toxoplasmosis (Morse, 1956; Tenter *et al*, 2000). *Toxoplasma gondii*, a zoonotic, heteroxenous obligate intracellular parasite has developed several potential routes of transmission within and between different host species (Levine, 1973; Ferguson *et al*, 1999). Common species of domestic and urban rats are chronic carriers of *Toxoplasma* and serve as a potential reservoir of infection to cats as well as other livestock animals (Wallace, 1973; Webster and MacDonald, 1995; Battersby, 1998). In the absence of cats, *T. gondii* can be maintained by vertical transmission in rats (Dubey, 1997a; Webster *et al*, 1994). Nevertheless, Wastling *et al* (2000) underscored the possibility of the natural life cycle of *T. gondii* via a cat-to-rodent-to-cat transmission which may indiscriminately involve infection of other warm blooded animals.

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In the Philippines, documented studies on toxoplasmosis are largely serologic in nature, in swine (Mendoza, 1974; Manuel and Tubongbanua, 1977; Marbella, 1980; Manuel, 1982), cats (Minervini, 1985; Dans, 2002), and in a few selected communities in Metro Manila, Mindoro and Leyte (Kawashima *et al*, 2000). Taking into account the wide distribution and abundance of rodents in environments close to human habitation (Fernando *et al*, 1985; Gratz, 1988), and their role as carriers/reservoir hosts of *T. gondii* (Galuzo, 1970; Fall, 1980), the present study sought to establish serologically the presence of *T. gondii* infection in *R. norvegicus* and *R. rattus mindanensis* caught in agricultural (AGR), commercial (COM), and residential (RES) sites in Dasmarinas, Cavite, Philippines.

MATERIALS AND METHODS

Using spring-door wire traps with food as bait, *Rattus* species were collected from AGR, COM and RES areas of the municipality of Dasmariñas, Cavite. *Rattus norvegicus* and *R. rattus mindanensis* were then identified (Fernando *et al*, 1985). Dasmariñas, Cavite is situated in the southern part of the Island of Luzon, Philippines, and is approximately 57 kms from Metro Manila, the National Capital Region (Research, Statistics, Monitoring and Evaluation Division, 2001). In the past ten years, the construction of commercial establishments and subdivisions has resulted in massive industrialization of the area and necessitated the conversion of a wide area of agricultural land.

Five ml of blood sample was extracted from each rat through venipuncture of the jugular vein. Blood was allowed to clot at RT for 30 minutes and centrifuged at 1,500 rpm for one minute. Sera were transferred into properly labeled tubes, stored in a refrigerator (4˚-8˚C), and were serologically processed within 24 hours post-collection.

Rat sera were assayed for the presence of anti-*T. gondii* Abs using the TOXOCELL AD Direct Agglutination Test Kit. The test kit contained a suspension of highly purified and concentrated *Toxoplasma (*Ags) used to determine the presence of IgM Abs (1:64-1:128) indicative of acute infection, and IgG Abs (≥1:256) indicative of chronic infection.

Serologic data generated per rat species and collection site were statistically analyzed using chi-square analysis and one-way analysis of variance (ANOVA) ($p \leq 0.05$).

RESULTS

A total of 157 rat sera were assayed for anti-*T. gondii* Abs. Eighty-seven (55.0%) were seropositive (sero$^+$), broken down as follows: 50 (60.0%) of the 83 *R. norvegicus,* and 37 (50.0%) of the 74 *R. rattus mindanensis* (Table 1). While serologic data suggest greater susceptibility of *R. norvegicus* to *T. gondii* relative to *R. rattus mindanensis*, statistical analysis showed insignificant association between parasite infectivity, rat species, and collection sites.

More male rats tested sero$^+$, except for *R. rattus mindanensis* caught in the COM site (Fig 1). Statistical analysis of gender-related data on toxoplasmosis in *R. norvegicus* and *R. rattus mindanensis* revealed insignificant association ($p>0.05$).

Comparison of acute and chronic cases across two rat species and three collection sites is summarized in Fig 2. Thirty-five (70.0%) of the sero$^+$ *R. norvegicus,* and 18 (49.0%) of sero$^+$ *R. rattus mindanensis* were chronic cases and registered anti-*T. gondii* Abs titer of 1:256-1:2048, and 1:256 1:1024, respectively (Table 2). All sero$^+$ COM-site *R. norvegicus* were chronically-infected.

DISCUSSION

The relatively high (>55.0%) number of sero$^+$ rats is consistent with earlier documented studies in domestic and wild rats (Webster and MacDonald, 1995; Battersby, 1998). Although more male rats tested sero$^+$ but there was insignificant association ($p>0.05$) of gender-related toxoplasmosis and this finding corroborates earlier findings in cats (Minervini, 1985) and humans (Lee *et al*, 2000), suggestive of the parasite's indiscriminate infectivity to both genders (Minervini, 1985; Dans, 2000).

Present findings are consistent with earlier reports that have identified domestic rats as chronic carriers of the tissue form of *Toxoplasma* (Wallace, 1973; Sasaki *et al*, 1976; Dubey *et al*, 1997b) and as a potent reservoir of infection to cats (Wallace, 1973).

The wide range of anti-*T. gondii* IgG titers assayed in the present study is suggestive of a difference in the status/persistence of *T. gondii* infection in rats surveyed vis-à-vis the frequency of their re-exposure to infection. Galuzo (1970) pointed out that the potential of animals to become reservoir hosts of *T. gondii* increases with re-exposure, where the host immune state is heightened with an increase in anti-*T. gondii* IgG titer. However, considering the continued proliferation of parasites even in the presence of high Abs titer, humoral-related immunity may still be insufficient to provide host protection (Stites *et al*, 1984).

Table 1
Number and serology of *R. norvegicus* and *R. rattus mindanensis* collected from agricultural (AGR), commercial (COM), and residential (RES) sites.

Collection site	No. sero+ *R. norvegicus*/ total no. assayed	No. sero+ *R. rattus mindanensis*/ total no. assayed (%))	Total no. sera (%)
AGR	20/34	13/28	33/62 (53.0)
COM	16/24	8/16	24/40 (60.0)
RES	14/25	16/30	30/55 (54.0)
Total sero+/total no. of sera species (%)	50/83 (60.0)	37/74 (50.0)	87/157 (55.0)

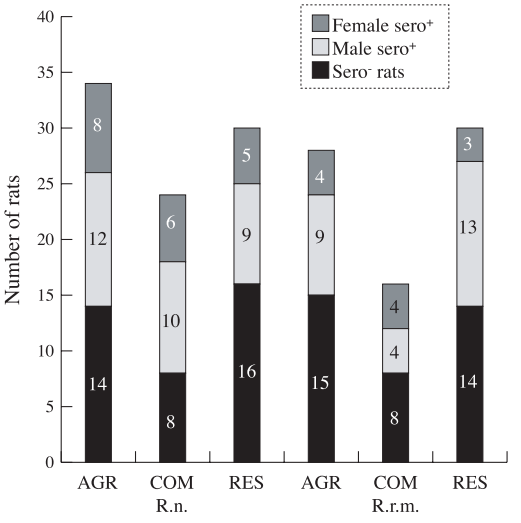

Fig 1- Gender-related *T. gondii* seropositivity differences in *R. norvegicus* (R.n.) and *R. rattus mindanensis* (R.r.m.) caught in agricultural (AGR), commercial (COM), and residential (RES) sites.

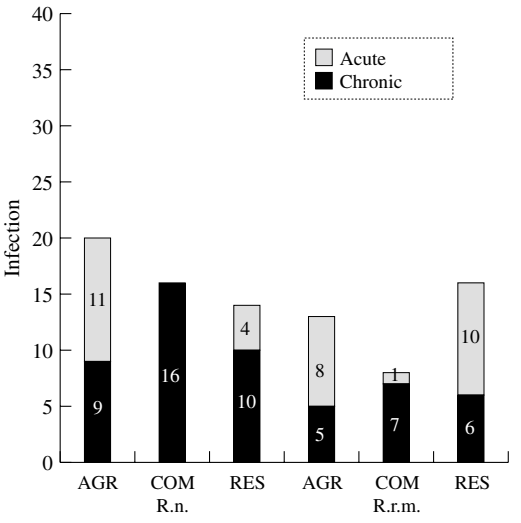

Fig 2- Comparison of acute and chronic cases of *T. gondii* infection in *R. norvegicus* and *R. rattus mindanensis* caught in AGR, COM, and RES sites; antibody titer (acute: 1:64-1:128; chronic: 1:256-1:2048).

Table 2
Toxoplasma gondii chronically-infected *R. norvegicus* and *R. rattus mindanensis* and titers of IgG antibodies.

Species	Collection site	Titer (IgG Abs) 1:256	1:512	1:1024	1:2048	Total chronic cases(%) Per site	Per species
R. norvegicus	AGR	3	4	2	0	9	35/50 (70.0)
	COM	5	7	3	1	16	
	RES	5	2	1	2	10	
R. rattus mindanensis	AGR	2	2	1	0	5	18/37 (49.0)
	COM	4	1	2	0	7	
	RES	1	3	2	0	6	
Total no. of rats per IgG titer (%)		20	19	11	3	53/87 (61.0)	

In conclusion, we have established serologically the presence of *T. gondii* infection in *R. norvegicus* and *R. rattus mindanensis* caught in the AGR, COM, and RES sites in Dasmarinas, Cavite, Philippines. To our knowledge, the present findings may represent the first documented study of *T. gondii* infection in rats in the country.

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