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Toxoplasma gondii infection in Philippines *Rattus* spp. confirmed through bioassay in *Mus musculus*

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ABSTRACT

The absence of any published work on *T. gondii* infection in rats in the Philippines has compelled us to embark on this present study. *Toxoplasma gondii* infection was serologically ascertained in 55.5% of *Rattus norvegicus* and *Rattus rattus mindanensis* caught in agricultural (AGR), commercial (COM) and residential (RES) sites at Dasmariñas, Cavite. Infection accounted for 60.0% in *R. norvegicus* and 50.0% in *R. rattus mindanensis*, with anti-*T. gondii* antibodies (Abs) titre ranging from 1:64 to 1:2048. Chronic infection (\geq 1:256 anti- *T. gondii* Abs) was detected in 53 (61.0%) rats. There was an insignificant association between parasite infectivity, rat species and collection sites. In mice, seropositivity was 65.0% (93/144), and tissue cysts were detected only among those exposed to brain tissue of RES caught *R. rattus mindanensis*. Although mouse infection was clearly inoculum dose related, the consistent higher infectivity of *T. gondii* parasite from AGR and COM relative to RES caught *Rattus* spp. and other biological parameters, such as clinical manifestations, parasite encystations and mortality, suggest intraspecific strain differences in *T. gondii* infecting the *Rattus* spp. populations. We are currently assessing the severity of histopathology in seropositive mice exposed to brain tissue of chronically infected *R. norvegicus* and *R. rattus mindanensis* across the three collection sites, and we hope to provide additional information that would help clarify present findings and observations. The present study represents the first confirmed report of *T. gondii* infection of rats in the country.

Key words: Toxoplasma gondii, Rattus spp., Philippines, serology, experimental mice

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Introduction

Toxoplasma gondii is an intracellular and obligate Coccidian zoonotic parasite that has developed several potential routes of transmission within and between different host species (DUBEY and BEATTIE, 1988). In the Philippines, studies on *T. gondii* infection are limited to pigs (MANUEL, 1982; MARBELLA, 1980; MANUEL and TUBONGBANUA, 1977; MENDOZA, 1974), in cats (DANS, 2002; MINERVINI, 1985) and humans (KAWASHIMA et al., 2000). Common species of domestic and urban rats have been reported as potential chronic carriers and reservoir of *T. gondii* infection to cats and other livestock animals (BERDOY et al., 2000; DEFEO et al., 2002; DUBEY et al., 1999; DUBEY and FRENKEL, 1998; WEBSTER and MACDONALD, 1995; WEBSTER, 1994), and humans (JONES et al., 2003; TENTER et al., 2001; HOVE and DUBEY, 1999). *Rattus norvegicus* and *Rattus rattus mindanensis* constitute two important and highly pestiferous species in the country (GRATZ, 1990; SANCHEZ et al., 1985) and in view of earlier studies suggesting their potential role in the spread of *T. gondii* infection to other animals and humans, we sought to determine the existence of this parasite species in *Rattus* spp. inhabiting agricultural, commercial and residential sites at Dasmariñas, Cavite, and confirmed the parasite through its bioassay in laboratory mice.

Materials and methods

Collection sites and identification of Rattus spp. Rats were collected from three different sites at Dasmariñas, Cavite, Philippines, as follows: a five hectare agricultural (AGR) land site; a commercial (COM) area largely occupied by a wet market close to where garbage is dumped and composted; and a residential (RES) site inhabited by more than 500 relocated families from Metro Manila. Rats were collected using spring-door wire traps that were set in the field or inside houses shortly before sunset and were checked the following morning. The rats caught based on their physical features and morphometrics (SANCHEZ et al., 1985), were identified as *R. norvegicus* (n = 83) and *R. rattus mindanensis* (n = 74).

Serological assay for anti-T. gondii Antibodies. Five mL of blood samples were extracted from each rat through venipuncture of the jugular vein, left to clot at room temperature for 30 min, and centrifuged at 1500 rpm for one min. Serum samples were assayed within 24 hr post-collection using Toxocell direct agglutination test kit (BIOKIT Manufacturing Company, Barcelona, Spain). Following the manufacturer's instruction, the assay was carried out using serum test dilutions of 1:4-1:64 and 1:128-1:2048. Anti-T. gondii antibody titres ranged from $\geq 1:164$ to 1:2048.

Preparation of brain tissue homogenate. Brain tissue was obtained from rats that registered $\geq 1:256$ anti- *T. gondii* Abs titre reflective of chronic infection (= presence of IgG Abs) and from seronegative (sero⁻) rats that were subsequently euthanized after the needed number of sero⁺ rats were obtained for experimental use. Brain tissue was processed (OMATA et al., 1994) with some modifications. Brain tissue was macerated in

0.95% mammalian phosphate buffer saline (PBS) solution, at a ratio of 1 gm brain tissue: 2.5 mL PBS. To preclude bacterial contamination, per 20 mL of brain tissue homogenate, 10 units of G-penicillin and 100 mg of streptomycin were added. Per rat species and per collection site, inoculum doses of 0.5 mL, 0.75 mL and 1.0 mL of brain tissue homogenate (BTH⁺) were prepared. For consistency of the injectable inoculum volume of 1.0 mL, 0.5 mL and 0.25 mL PBS were added to 0.5 and 0.75 mL BTH⁺, respectively. For the control groups, inoculum dose of 1.0 mL brain tissue homogenate prepared from sero⁻rats (BTH⁻), and 1.0 mL PBS were likewise prepared.

Mouse inoculation, monitoring of the establishment of infection and other biological parameters. Mice (ICR strain), 6-7 weeks old and weighing 20-25 gm were procured from the Animal Physiology Laboratory, College of Veterinary Medicine (CVM), University of the Philippines, Los Baños (UPLB), Laguna. Mice were acclimatized under laboratory conditions for one week prior to use and were maintained according to the Philippine Association for Laboratory Animal Science (PALAS) Code of Practice for the Care and Use of Laboratory Animals in the Philippines (2002).

Per rat species as the source of BTH⁺, a total of 72 experimental mice was used. Twentyfour mice each were inoculated BTH⁺ from agricultural (BTH⁺/AGR), commercial (BTH⁺/ COM) and residential (BTH⁺/RES) inhabiting rats. For the control, 18 mice each were inoculated 1.0 mL BTH⁻ and 1.0 mL PBS. Brain tissue suspension was intraperitoneally administered at the lower right quadrant of the abdomen.

Mouse serum samples from experimental and control mice were assayed for anti-*T. gondii* Abs weekly, for four weeks post-inoculation (p.i.) using the Toxocell latex agglutination test (LAT) (BIOKIT Manufacturing Company, Barcelona, Spain). At 2 and 5 days p.i. and prior to euthanization, the presence of *T. gondii* parasites were examined in thick and thin blood smears prepared from 1-2 drops of blood samples obtained from nipping off a small piece of the mouse tail, and in peritoneal exudates using the lavage method (OMATA et al., 1994). Smears were stained with Giemsa (BRUCE-GREGORIOS, 1974). Per week and for four weeks p.i., per treatment two sero⁺ mice were sacrificed for use in the histopathogical evaluation of vital organs. *Toxoplasma gondii*-associated physical and behavioral changes, mouse body mass before and after inoculation and mortality were monitored. Vital organs of dead experimental and control mice were likewise removed for the purpose of histopathological assessment.

Statistical analysis. Serologic data in rats were analyzed using chi-square analysis and one-way analysis of variance (ANOVA) (P \ge 0.05). Mouse serolog y and body mass changes were analyzed using Student's *t*-test and ANOVA at P \ge 0.05, and P \ge 0.01, respectively.

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Results

Eighty-seven (55.0%) of the rat serum samples were positive for anti-T. gondii Abs at titres 1:64-1:2048: 60.0% (50/83) in R. norvegicus, and 50.0% (37/74) in R. rattus mindanensis. A total of 53 rats (61.0%) registered ≥1:256 anti- T. gondii Abs titre, comprising 70.0% (35/50) in *R. norvegicus* (Abs titre: 1:256-1:2048), and 49.0% (18/37) in R. rattus mindanensis (Abs titre: 1:256-1:1024). Analysis of serological data showed insignificant (P>0.05) association between parasite infectivity, rat species and collection sites.

Table 1. Comparison of mean body mass change for four weeks post-inoculation in mice exposed to brain tissue homogenate (BTH⁺) of seropositive R. norvegicus and R. rattus mindanensis.

Dosage (mL)	R. norvegicus		R. rattus mindanensis	
	Range	Mean mass change $(gm) \pm SD.$	Range	Mean mass change $(gm) \pm SD$
0.5	0.93-2.30	^a 1.94 ± 0.328	1.33-2.20	$a 1.93 \pm 0.181$
0.75	1.08-2.60	^a 1.89 ± 0.296	0.55-2.30	^a 1.82 ± 0.456
1.0	0.86-2.60	^b 1.55 ± 0.475	1.30-2.30	^a 1.70 ± 0.293
Mean mass/species	0.85-2.60	° 1.81 ± 0.399	0.55-2.30	° 1.83 ± 0.337
significant $a - b (P < 0.01)$ significant $a - a, c - c (P > 0.01)$				

significant °(P≤0.01), significant ^e(P>0.01)

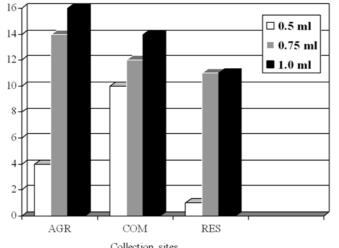
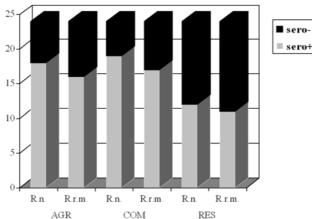


Fig. 1. A. Comparison of the total number of sero⁺ mice four weeks post-inoculation with brain tissue homogenate (BTH⁺) from chronically infected R. norvegicus (R.n.) and R. rattus mindanensis (R.r.m.). A. According to rat collection sites (AGR, COM and RES). All control (BTH and PBS inoculated) mice were seronegative.



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Fig. 1. B. Comparison of the total number of sero⁺ mice four weeks post-inoculation with brain tissue homogenate (BTH⁺) from chronically infected *R. norvegicus* (R.n.) and *R. rattus mindanensis* (R.r.m.). B. Showing influence of different inoculum dose on the establishment of *T. gondii* infection. Bars represent total number of mice confirmed positive for anti-*T. gondii* antibodies at week 4 post-inoculation.

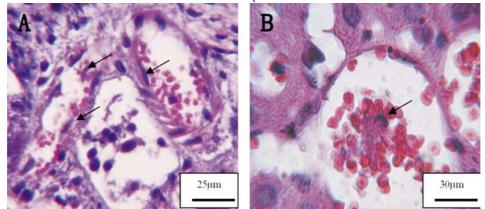


Fig. 2. Tissue sections of infected mice showing tachyzoites (arrows) in cardiac veins (A) and hepatic vein (B). Note ruptured hepatic portal vein. H&E stain.

Ninety-three (65.0%) of the 144 experimental mice were confirmed positive for anti-*T.* gondii Abs: 49 (68.0%) exposed to *R. norvegicus* (R.n.)-BTH⁺, and 44 (61.0%) to *R. rattus* mindanensis (R.r.m.)-BTH⁺ (Fig. 1A), the difference of which was found to be insignificant (P>0.05). However, there were significantly (P \leq 0.05) more sero ⁺ mice exposed to BTH⁺ of AGR and COM inhabiting *Rattus* spp., as compared to RES type of rats. The dose related

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influence on the establishment of infection was likewise significant, particularly between the 0.5 mL and higher doses of 0.75-1.0 mL BTH⁺ (Fig. 1B).

At week-1 p.i. tachyzoites in blood smears and peritoneal exudates were detected in 19.0% of mice exposed to R.r.m-BTH⁺, and only in 5.0% of mice inoculated R.n.-BTH⁺ (Fig. 2). All the control mice, including two BTH⁻ inoculated dead mice, were negative for parasites. Sero⁺ mice manifested lethargy, ruffled hair, head and neck extension, and hunching. About 18 (19.0%) of the sero⁺ mice were clinically normal. As early as week-3 p.i. tissue cysts were found only in the diaphragm, heart and brain of mice exposed to BTH⁺ of *R. rattus mindanensis* (Fig. 3).

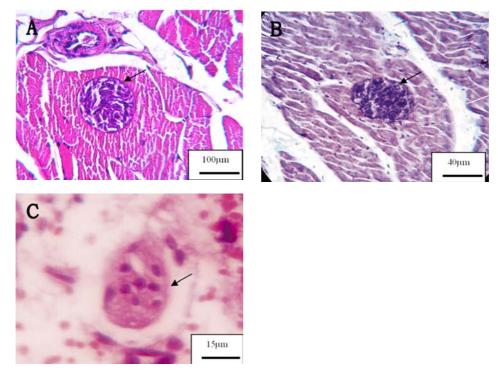
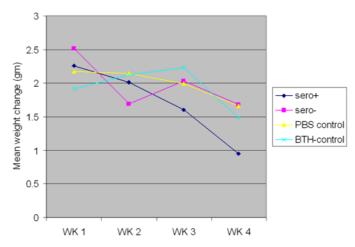


Fig. 3. Tissue cysts (arrow) in the diaphragm (A), heart (B) and brain (C) tissues of infected mice post inoculation with brain tissue of seropositive residential dwelling *R. rattus mindanensis*. H&E stain.



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Fig. 4. Weekly mean body mass change in experimental and control mice. R (range). MWG (mean mass change). Sero⁺ (r: 0.55-2.60 gm: mwg: 1.71 ± 0.433); Sero⁻ (r:1.78-2.23 gm: mwg:1.98 ± 0.11); PBS control (r:1.79-2.20 gm; mwg: 1.99 ± 0.10); BTH⁻ control (r: 1.65-2.15 gm; mwg: 1.94± 0.31). Significant (P<0.01) difference between sero⁺, and sero⁻, BTH⁻ and PBS control groups

Sero⁺ mice registered significantly (P \leq 0.01) low mean body mass change (mbwc) relative to sero⁻ and control mice (Fig. 4). While the difference in mbwc between sero⁺ mice inoculated with R.n.-BTH⁺ and R.r.m.-BTH⁺ was insignificant, it was appreciably lower in mice exposed to 1.0 mL R.n.-BTH⁺ (Table 1). Of the 10 dead sero⁺ mice (10.8%), 7 were exposed to as low as 0.5 mL BTH⁺ of *R. norvegicus* in contrast to only 3 mice that were exposed to 0.75-1.0 mL BTH⁺ of *R. rattus mindanensis*. Moreover, 8 of these dead mice were infected with *T. gondii* parasites obtained from AGR and COM dwelling rats. Considering that sero⁺ mice had to be sacrificed each week for four weeks for purposes of pathological evaluation, mortality could have been higher among mice exposed to R.n.-BTH⁺.

Discussion

Present serological data are collaborated by earlier reports of *T. gondii* infection ranging between 23.3% to 41.7% in *R. norvegicus* (FRENKEL et al., 1995; MORSY et al., 1994; WEBSTER, 1994), and 11.6-13.0% in *R. rattus* (EL-SHAZLY et al., 1991; FRENKEL and RUIZ, 1981; BURRIDGE et al., 1979). However, we obtained a higher percent infection and with greater than 50.0% of the sero⁺ rats having registered >1:256 anti-*T. gondii* Abs titre. The impoverished condition in the study sites inhabited largely by relocated poor

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families, evidenced by human congestion, poor sanitation and abundance of stray cats and dogs (personal observations), may have contributed to the higher infection rate in rats in the present study. WEBSTER (1994) had demonstrated the perpetuation of *T. gondii* infection, particularly within wild rat populations without the sympatric presence of cats, and proposed congenital transmission as the predominant transmission route, concluding that rats represent significant and persistent wildlife intermediate hosts of *T. gondii*.

Physical and behavioral manifestations in sero⁺ mice, accompanied by stunted growth, are similar to earlier observations in *T. gondii*-infected rats and mice (WEBSTER et al., 1994). Interestingly, 19.0% of the sero⁺ mice exhibited unconvincing *T. gondii* related manifestations of infection, suggestive of an asymptomatic latent toxoplasmosis where pathological changes are negligible owing to parasite encystations in muscles or brain, or a case of asymptomatic acute toxoplasmosis where tachyzoites proliferation is shortened and, with immunity, developing before any significant changes are produced (KATSUBE et al., 1968; JACOBS and MELTON, 1957). Dose related influence on the establishment of infection in mice was evident. However, the consistent higher infectivity of parasites from *R. norvegicus* and *R. rattus mindanensis* caught in the AGR and COM sites suggests a *T. gondii* isolate different from that of the RES dwelling rat population. Also, the detection of tissue cysts in mice exposed to 0.75-1.0 mL of RES-*R. rattus mindanensis* as early as week-3 p.i. is indicative of a less virulent or avirulent parasite strain (BOOTHROYD and GRIGG, 2002).

The infectivity of bradyzoites present in cysts of chronically infected rats relative to tachyzoites (DUBEY et al., 2001) may have influenced the high infection rate we obtained. Likewise, the oral route of *T. gondii* administration has been reported to cause mortality in laboratory mice even when exposed to either low virulent or avirulent strain, and to as high as 60.0% when exposed to the virulent Beverly strain (FRENKEL, 1974). We reckon that the 10.8% mortality obtained in the present study may have been influenced by the intraperitoneal mode of inoculation used, albeit mortality may have been higher in the absence of euthanization for purposes of histopathological evaluation.

In conclusion, we have serologically ascertained *T. gondii* infection in *Rattus* spp. inhabiting AGR, COM and RES sites at Dasmariñas, Cavite, and confirmed the presence of *T. gondii* parasites through its bioassay in mice. Present data suggest a difference in the strain(s) of *T. gondii* infective to *R. norvegicus* and *R. rattus mindanensis* and between the AGR/COM and RES rat populations. We are currently assessing the extent and severity of histopathology in infected mice exposed to BTH⁺ of *Rattus* species across the three collection sites, and we hope to document additional information that would help clarify present findings and observations. The present work represents the first confirmed report of *T. gondii* infection of rats in the country.

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SAŽETAK

Istraživanje je potaknuto nedostatkom objavljenih radova o invazijama vrstom *T. gondii* u štakora na Filipinima. Invazija tom vrstom praživotinje serološki je potvrđena u 55.5% štakora (*Rattus norvegicus* i *Rattus rattus mindanensis*) ulovljenih u poljoprivrednim, trgovačkim i stambenim područjima u Dasmariñas, Cavite. Invazija je ustanovljena u 60% štakora *R. norvegicus* i 50% *R. rattus mindanensis*, s titrom protutijela od 1:64 do 1:2048. Kronična invazija (titar protutijela \geq 1:256) ustanovljena je u 53 (61,0%) štakora. Povezanost između parazitskih invazija, vrste štakora i mjesta s kojih su bili sakupljeni nije bila značajna. U 65,0% (93/144) miševa utvrđen je pozitivan serološki nalaz, a tkivne ciste otkrivene su jedino u miševa invadiranih moždanim tkivom štakora *R. rattus mindanensis* ulovljenih u nastanjenim područjima. Iako je invazija miševa ovisila o inokuliranoj dozi, veća invazivnost parazita *T. gondii* podrijetlom iz poljoprivrednih i trgovačkih područja u odnosu na stambena područja, kao i drugi biološki pokazatelji poput kliničkih znakova, parazitske encistacije i smrtnost upozoravaju na razlike unutar vrste *T. gondii* kojom je invadirani stakorska populacija. Trenutno se istražuju histopatološke promjene u serološki pozitivnih miševa koji su invadirani tkivom mozga kronično invadiranih stakora *R. norvegicus* i *R. rattus mindanensis* s tri lokacije. Ovo istraživanje predstavlja prvi potvrđeni nalaz invazije štakora parazitom *T. gondii* u zemlji.

Ključne riječi: Toxoplasma gondii, Rattus spp., Filipini, serologija, pokusni miševi