SEROPREVALENCE OF *TOXOPLASMA GONDII* IN RODENTS FROM VARIOUS LOCATIONS IN PENINSULAR MALAYSIA

Y Normaznah, MA Noor Azizah, MI Mohd Azuan, I Latifah, S Rahmat and M Amal Nasir

Medical Research Resource Centre, Institute for Medical Research, Kuala Lumpur, Malaysia

Abstract. Numerous studies have reported the prevalence of toxoplasmosis among Malaysians and various domestic animals; but there is paucity of information on its prevalence among rodents which could potentially contribute to the transmission of *Toxoplasma gondii* in both domestic and sylvatic fauna. Five hundred twenty-six rodents were captured from six locations in Malaysia and identified to species. Serum samples were collected from these rodents and tested for *T.gondii* antibodies using an immunofluorescent antibody test (IFAT). *T.gondii* antibodies were found in 5.9% (31/526) of the tested samples. Most of the positive antibodies were from commensal rats: *Rattus exulans* (9/64, 14.0%), *Rattus argentiventer* (2/8, 25%), *Rattus rattus diardii* (10/166, 6.0%) and *Rattus tiomanicus* (6/215, 2.7%). Only two of the forest rats were positive: *Maxomys rajah* (1/9, 11.1%) and *Rattus bowersi* (1/12, 8.3%). Eighteen point one percent of ground squirrels (*Tupaia glis*) tested (2/11) were positive for antibodies. The highest antibodies titer (1:1024) was found in *Rattus exulans* followed by *T.glis* (1:256). Sabak Bernam, Selangor had the highest prevalence (10.8%) followed by Baling, Kedah (5.0%) and Bagan Terap, Selangor (4.0%). None of the serum samples of rodents collected from Gua Musang, Kelantan; Jasin, Malacca; or Labis, Johor were positive. Our study reports for the first time the serologic prevalence of *T.gondii* antibodies among rodents in Peninsular Malaysia. Further studies are needed to confirm *T.gondii* infection among wild rodents, such as a bioassay, to assess their potential role in transmission of the parasite.

Keywords: *Toxoplasma gondii,* seroprevalence, rodents, Malaysia

INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan that infects most species of warm blooded vertebrates, including rodents (Dubey and Beattie, 1988; Dubey and Frenkel, 1998). Cats are the definitive hosts of *T.gondii* (Dubey *et al*, 1970). Infected cats shed *T.gondii* oocysts in feces, contaminating the environment (Dubey *et al*, 1970). Rodents may become infected by ingesting soil, vegetation or water contaminated with *T.gondii* oocysts shed in cat feces (Dubey, 1996). *T.gondii*-infected rats may serve as a reservoir of infection for animals such as pigs, dogs and cats (Dubey and Frenkel, 1998). In humans, the parasite causes toxoplasmosis, which is

Correspondence: Y Normaznah, Medical Research Resource Centre, Institute for Medical Research, Jalan Pahang, Kuala Lumpur 50588, Malaysia.
E-mail: maznah@imr.gov.my; dr_normaznay@yahoo.com

generally acquired by ingesting sporulated oocysts in contaminated food or water, or by consuming insufficiently cooked meat containing tissue cysts (Dubey and Beattie, 1988). It may cause a life-threatening opportunistic infection, particularly in immunocompromised patients and developing fetuses (Dubey and Beattie, 1998). Toxoplasmosis is reported to have high prevalence among Malaysians and others in Southeast Asian (Nissapatorn *et al*, 2005; Nissapatorn, 2007). In Malaysia, numerous studies have been conducted to determine the seroprevalence of *T.gondii* in human and domestic animals (Yahaya, 1991; Nissapatorn and Khairul Anuar, 2004, Nissapatorn *et al*, 2005), however, there is limited information regarding its prevalence in rodents. The aim of this study was to determine the prevalence *T.gondii* antibodies in wild rodents caught from various locations in Peninsular Malaysia.

MATERIALS AND METHODS

Rodent trapping

Wild field rodents were trapped in various locations in Malaysia: a rural rubber tree plantation (Baling, Kedah), a forest area (Gua Musang, Kelantan), a palm oil and pineapple plantation (Jasin, Melaka), a palm oil plantation (Labis, Johor) and a rice field (Sabak Bernam dan Bagan Terap, Selangor). Rodents were trapped using rectangular wire cages. Ripe banana, coconut and salted fish were used as bait. The traps were checked every morning. The trapped rodents were collected and then euthanized with chloroform. Rodents were identified to species based on physical and morphological features using the identification keys and illustrations developed by Harrison and Quah (1962); Medway (1983) and Junaidi *et al* (1985).

Immunofluorescent antibody test

About 0.2 ml of blood was collected from each rodent into plain tubes via cardiac puncture. The tubes were transported to the laboratory and then centrifuged at 5,000*g* for 10 minutes. Serum was collected into storage tubes and stored at -20°C until used.

The serum samples were then tested for antibodies against *T.gondii* with an immunofluorescent antibody test (IFAT) as described by Hakim *et al* (1994). The slides were examined at x200 under an immunofluorescent microscope (Nikon, Tokyo, Japan). An IgG antibody titer ≥1:64 and IgM antibody titer ≥1:4 were considered positive. Positive and negative controls were also used.

RESULTS

A total of 526 rats were collected and sampled from 6 different locations in Peninsular Malaysia: rice fields in Sabak Bernam (*n*=202) and Bagan Terap (*n*=25), Selangor; a rubber plantation in Baling, Kedah (*n*=159); a forest in Gua Musang, Kelantan (*n*=85); a palm oil and pineapple plantation in Jasin, Malacca (*n*=15) and a palm oil plantation in Labis, Johor (*n*=40) (Table 1). Fourteen species of rats were identified. The most common species found was *Rattus tiomanicus* (40.9%) followed by *Rattus rattus diardii* (31.6%) and *Rattus exulans* (12.2%). *Rattus tiomanicus* was the most common species found in Gua Musang Kelantan (77.6%), Baling Kedah (56.6%), Jasin Melaka (53.3%) and Labis Johor (90%). *Rattus rattus diardii* was the most common species found in Sabak Bernam (61.9%) and Bagan Terap (80%), Selangor. *Rattus exulans* was mostly found in Sabak Bernam, Selangor.

Of the 526 samples analyzed, 31(5.9%) were positive for *T.gondii* antibodies by

Table 1
Species of rodents by location.

Species of rodents	Baling, Kedah	Sabak Bernam, Selangor	Bagan Terap, Selangor	Gua Musang, Kelantan	Jasin, Melaka	Labis, Johor	Total no of rodents according to species
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Rattus tiomanicus	90 (56.6)	13 (6.4)	2 (8.0)	66 (77.6)	8 (53.3)	36 (90.0)	215 (40.9)
Rattus rattus diardii	16 (10.1)	125 (61.9)	20 (80.0)	2 (2.4)	3 (20.0)	0	166 (31.6)
Rattus exulans	1 (0.6)	58 (28.7)	1 (4.0)	0	4 (26.7)	0	64 (12.2)
Sundamys muelleri	5 (3.1)	0	0	9 (10.6)	0	0	14 (2.7)
Rattus surifer	9 (5.7)	0	0	0	0	0	9 (1.7)
Niniventer criomoriven	9 (5.7)	0	0	2 (2.4)	0	0	11 (2.1)
Tupaia glis	7 (4.5)	0	0	0	0	4 (10.0)	11 (2.1)
Rattus bowersi	12 (7.7)	0	0	0	0	0	12 (2.3)
Maxomys rajah	8 (5.1)	0	0	1 (1.2)	0	0	9 (1.7)
Rattus argentiventer	0	6 (3.0)	2 (8.0)	0	0	0	8 (1.5)
Maxomys surifer	0	0	0	3 (3.5)	0	0	3 (0.6)
Maxomys whiteheadi	1 (0.6)	0	0	1 (1.2)	0	0	2 (0.4)
Leopoldamys sabanus	0	0	0	1 (1.2)	0	0	1 (0.2)
Rattus muelleri	1 (0.6)	0	0	0	0	0	1 (0.2)
Total	159 (100)	202 (100)	25 (100)	85 (100)	15 (100)	40 (100)	526 (100)

IFAT (Table 2). The rats with the highest prevalence of *T.gondii* antibodies was found in Sabak Bernam (10.8%) followed by Baling, Kedah (5.0%) and Bagan Terap, Selangor (4.0%). None of the rodents caught in Gua Musang, Kelantan; Jasin, Melaka or Labis, Johor were positive for *T.gondii* antiboies.

The rat species with the highest prevalence of *T.gondii* antibodies was in *Rattus argentiventer* (2/8, 25%) followed by *Rattus exulans* (9/64, 14.0%), *Maxomys rajah* (1/9, 11%), *Rattus bowersi* (1/12, 8.3), *Tupaia glis* (2/11, 9%), *Rattus rattus diardii* (11/166, 6.6%) and *Rattus tiomanicus* (6/215, 4.6%) (Table 3). The following species were negative for *T.gondii* antibodies: *Sundamys muelleri*, *Rattus surifer*, *Niniventer criomoriven*, *Maxomys surifer*, *Maxomys whiteheadi*, *Leopoldamys sabanus* and *Rattus muelleri*.

DISCUSSION

In Malaysia, there is limited information about *T.gondii* infection among rodents. A study by Nimir and Linn (2011) reported 3% of rats captured at a wet market in the capital city of Kuala Lumpur had *T.gondii* cysts in the brain on microscopic examination of the tissue sections. A study by Fazly *et al* (2013) of zoonotic parasites found 1 of 5 squirrels tested was positive for toxoplasmosis on histology. There are no published reports of the seroprevalence of *T.gondii* among rodents in Malaysia. The overall prevalence of *T.gondii* antibodies among rodents tested in this study was 6.0%. The species with the highest percentage of positivity was found in *Rattus argentiventer* (25%) followed by *Rattus exulans* (17.1%), *Rattus bowersi* and *Maxomys rajah*

Table 2
Prevalence of *T.gondii* antibodies in rodents by location.

Locality	Species	No. tested	Positive IgG only	Positive IgM only	Positive both IgG and IgM	Percent positive
Baling, Kedah						
	Rattus tiomanicus	90	1	2	0	3.3
	Rattus rattus diardii	16	0	0	0	0
	Rattus bowersi	12	0	1	0	8.3
	Rattus surifer	9	0	0	0	0
	Rattus exulans	1	0	0	1	100
	Sundamys muelleri	5	0	0	0	0
	Maxomys rajah	8	0	1	0	12.5
	Niniventer criomoriven	9	0	0	0	0
	Tupaia glis	7	2	0	0	28.5
	Maxomys whiteheadi	1	0	0	0	0
	Rattus muelleri	1	0	0	0	0
Sub total		159	3	4	1	5.0
Bagan Terap, Selangor						
	Rattus rattus diardii	20	0	1	0	5.0
	Rattus tiomanicus	2	0	0	0	0
	Rattus argentiventer	2	0	0	0	0
	Rattus exulans	1	0	0	0	0
Sub total		25	0	1	0	4.0
Sabak Bernam, Selangor						
	Rattus rattus diardii	125	2	3	4	7.2
	Rattus tiomanicus	13	0	2	1	23.0
	Rattus exulans	58	5	1	2	13.7
	Rattus argentiventer	6	1	1	0	33.0
Sub total		202	8	7	7	10.8
Total		526[a]	11	12	8	5.9 (31/526)

[a]The total included samples from Labis, Johor (*n*=40), Jasin, Malacca (*n*=15) and Gua Musang, Kelantan (*n*=85). The samples were all negative.

(11.1%), *Tupaia glis* (9.0%), *Rattus rattus diardii* (6.6%) and *Rattus tiomanicus* (4.6%).

A review by Dubey and Frankel (1998) of the serologic prevalences of *T.gondii* in rats from different countries found the prevalences ranged from 1% to 70% using various different serological methods. The authors pointed out the difficulty of comparing findings among the studies due to different diagnostic methods used. The prevalence of *T.gondii* antibodies in rodents in this study is similar to the prevalence of 4.6% reported from Thailand (Jittapalapong *et al*, 2011) but lower than 55.5% reported from the Philippines (Salibay and Claveria, 2005). Zhang *et al* (2004) found *T.gondii* antibodies among 26% of field mice tested in Yuanjiang, Hunan Province, People's Republic of China. Dabritz *et al* (2008) found 17% of wild rodents tested from central coastal California were positive serologically. Dubey *et al* (2006) found

Table 3
Toxoplasma gondii antibody positive by IFAT by rodent species.

Species of rodents	Number of rodents tested	Number of positive rodents (%)
Rattus tiomanicus	215	6 (2.7)
Rattus rattus diardii	166	10 (6.0)
Rattus exulans	64	9 (14.0)
Sundamys muelleri	14	0
Rattus surifer	12	0
Niniventer criomoriven	11	0
Tupaia glis	11	2 (18.1)
Rattus bowersi	12	1 (8.3)
Maxomys rajah	9	1 (11.1)
Rattus argentiventer	8	2 (25.0)
Maxomys surifer	3	0
Maxomys whiteheadi	2	0
Leopoldamys sabanus	1	0
Rattus muelleri	1	0
Total	526	31 (5.9)

only 0.8% of rats tested in Grenada, West Indies were positive for *T.gondii*.

The rats trapped in our study were mostly commensal or forest rats. The commensal rats included *Rattus rattus diardii*, *Rattus tiomanicus* and *Rattus exulans* and the forest rats included *Sundamys muelleri*, *Maxomys rajah*, *Maxomys whiteheadi*, *Niniventer criomoriven* and *Leopoldamys sabanus*. The number of commensal rats recovered was higher than the number of forest rats recovered. The most common species captured was *Rattus tiomanicus*; 2.7% were positive for *T.gondii* antibodies. *Rattus tiomanicus* (Malaysian field rat) normally inhabits secondary forests and is a serious pest in oil palm plantations (Wood and Chung, 1990). The species has adapted to the environmental conditions caused by humans, such as deforestation and land development, and has thrived to become the dominant species (Lim, 1974a, b). The second most common species recovered was *Rattus rattus diardii,* of whom 6.6% were positive for *T.gondii* antibodies. *Rattus rattus diardii* is one of the principal domestic rats in urban Malaysia (Zahedi *et al*, 1984; Nimir and Linn, 2011; Paramasvaran *et al*, 2013) and is commonly found in fields and near human dwellings (Harrison and Quah, 1962). In our study, although the number of *Rattus exulans* specimen captured was lower than the above mentioned species, the percent positive for *T.gondii* antibodies was higher (14%). Jittapalapong *et al* (2011) reported a low prevalence of 1.3% among *Rattus exulans* in Thailand. *Rattus exulans* survives in various habitats, such as scrub and forest with adequate food supplies and shelter (Taylor, 1975). *Rattus exulans* also lives exclusively inside and around houses and is more likely to be exposed to oocysts shed by infected cats (Jittapalapong *et al*, 2011). The other species of the genus *Rattus* trapped was *Rattus bowersi*. The species is bigger in size and tends to inhibit forests (Paramasvaran *et al*, 2013); 8.3% were positive for *T.gondii* antibodies. The rice field rat, *Rattus argen-*

tiventer was only captured from rice fields in Sabak Bernam and Bagan Terap in the state of Selangor. Despite the low numbers captured, the prevalenve of *T.gondii* found in this species was far higher than the other aforementioned *Rattus* species. *T.gondii* antibodies were detected in the forest rat *Maxomys rajah* (11.0%) but the other species were all negative. Infected forest rats can potentially serve as sources of transmission in a wild sylvatic cycle.

Eleven *Tupaia glis* (ground squirrel) specimens belonging to the family Sciuridae were trapped in the study. Two of the 7 (28.5%) trapped in Baling were positive for *T.gondii* antibodies, but none of those trapped in Labis, Johor was positive. The overall seroprevalence of *T.gondii* antibodies among squirrels found in this study (18%) was higher than the seroprevalece found in squirrels from California (13.6%), Kansas (18%) and Thailand (7.5%) (Smith and Frenkel 1995; Dabritz *et al*, 2008; Jittapalapong *et al*, 2011). The squirrels may have acquired the infection through ingestion of oocysts shed by stray cats that frequent the area.

T.gondii antibodies were detected in 3 locations: Baling in Kedah, Sabak Bernam and Bagan Terap, both in Selangor. No positive rodents were detected in Gua Musang, Kelantan; Jasin, Malacca or Labis, Johor. Trapping sites in Sabak Bernam and Bagan Terap in Selangor were rice fields near human settlements. Only commensal rats were recovered at this site. *Rattus rattus diardii*, *Rattus exulans*, and *Rattus tiomanicus*, rice field rats, *Rattus argentiventer* were trapped in this area. Various percentages of commensal rats in Sabak Bernam were positive for *T.gondii* antibodies. The study site in Baling, Kedah was a rubber tree plantation. Rural and agricultural areas with a fairly low density of humans may be more suitable for commensal and forest rats. The prevalence of *T.gondii* antibodies in Baling, Kedah (5.0%) was not much different from that in Bagan Terap, Selangor (4.0%), but the prevalence in Sabak Bernam, Selangor was twice that of Baling, Kedah (10.8% *vs* 5.0%).

The trapping site in Gua Musang, Kelantan was mostly forest, distant from human settlements. More forest rats were trapped in that area than commensal rats (Table 3). *T.gondii* antibodies were not detected in any specimen from that habitat. Jasin, Malacca and Labis, Johor study sites were palm oil and pineapple plantations. Only commensal rats were trapped in those areas, and all were negative for *T.gondii* antibodies. There was a marked difference in the number of rodents trapped at these different locations. It is not known if the low trap success at certain sites was related to cat predation or rodent control efforts.

Higher antibody titers were observed among rodents from Sabak Bernam (1:64 to 1:1024) and higher titers (1:256 to 1:1024) were found in *Rattus exulans* specimens. The highest antibody titers seeen in rodents from Baling, Kedah was 1:256 and in a ground squirrel (*T.glis*). It is unknown if this relates to the abundance of stray cats in the survey area or different susceptibilities of the rodent species. Previous studies of swine farms found the prevalence of *T.gondii* infection among mice was associated with higher mean cat densities (Smith *et al*, 1992; Dubey *et al*, 1995).

This is the first report of the serologic prevalence of *T.gondii* antibodies among wild rodents in Peninsular Malaysia. Of 526 samples, 5.9% were found positive for *T.gondii*. Since only a small proportion of the serologically positive rats were actually infected (Dubey and Frenkel, 1998), further studies need to be conducted using a bioassay to evaluate the possible

role of these rodents in the transmission and maintenance of the *T.gondii* life cycle.

ACKNOWLEDGEMENTS

The authors thank the Director General of Health, Ministry of Health, Malaysia for his permission to publish the paper. We also thank the Director of the Institute for Medical Research for her support and encouragement to publish this paper. This study was approved by the Animal Use Committee, IMR and financially supported by the National Institute of Health (NIH) Research Fund, Ministry of Health Malaysia.

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