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Rickettsiae exposure related to habitats of the oriental house rat (*Rattus tanezumi*, Temminck, 1844) in Salaya suburb, Thailand

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ABSTRACT

Rickettsial zoonotic diseases, in particular scrub typhus, murine typhus, and tick typhus, are caused by *Orientia tsutsugamushi*, *Rickettsia typhi*, and *Rickettsia honei* infections. Rickettsiae exposure is typically related to a rodent host in various habitats of marginal regions, including between rural areas and communities such as the Salaya suburb. This allows the oriental house rat (OHR), a highly adaptive species, to live in close proximity to the community and possibly introduce rickettsial diseases. To understand rickettsial exposure in the OHR from different habitats, knowledge of disease exposure can serve as baseline information for disease management and prevention. A total of 185 OHRs from three unrelated habitats were assessed using a standard indirect immunofluorescence assay (IFA) for specific antibody reaction to *O. tsutsugamushi*, *R. typhi*, and *R. honei*. The presence of antibody in the OHR to rickettsiae, either scrub or murine typhus, was associated with the habitat, whereas tick typhus had general exposure. This finding shows the OHR to be a potential reservoir host for rickettsial diseases along the borders of geographic regions in the suburban environment.

1. Introduction

Rodent-borne rickettsiae are intracellular microorganisms that are mediated by rodent species as their natural reservoir host and transmitted by arthropod vectors (Zain et al., 2015). In particular, scrub, murine, and tick typhus infections are caused by individual infectious rickettsiae, *Orientia tsutsugamushi*, *Rickettsia typhi*, and *Rickettsia honei*, which are transmitted from the rodent reservoir host to other mammals by an individual arthropod vector, including chigger mites (*Leptotrombidium deliense*), rat fleas (*Xenopsylla cheopis*) and hard ticks (*Ixodes granulatus*), respectively (Blasdell et al., 2015; Graves and Stenos, 2003). In Thailand, sporadic rickettsial infections have been associated with the presence of either rodent exposure or ectoparasitic vectors (Lerdthusnee et al., 2008; Watt and Parola, 2003). The Armed Forces Research Institute of Medical Sciences (AFRIMS) reported that the sporadic incidence of scrub typhus in humans was 7.11 and 5.82 individuals per 100,000 populations during 2018 and 2019, respectively (Tabprasit, 2019). This incidence presents a problem for public health as a result of the associated illness, medical treatment costs, and subsequent absences from duty and furthermore results in economic loss. In the fact of humans are an accidental host of scrub typhus infection after being bitten by a vector, namely, chigger mites (Kuo et al., 2012; Watt and Parola, 2003). Furthermore, the abundance of a synanthropic rodent species is consistent for one highly adaptable species in particular, the oriental house rat (OHR: *Rattus tanezumi*) (Heaney and Molur, 2016), which results in its being typically found around residential areas and agricultural fields. This rat can expose and harbor multiple pathogens, particularly numerous helminth species, *Trypanosoma* spp., *Leptospira* spp., *Bartonella* spp., Hantaviruses (Chaisiri et al., 2015; Johansson et al., 2010; Kocher et al., 2015; Loan et al., 2015; Plyusnina et al., 2009), and a further high diversity of arthropod ectoparasites, some of which play the role of vector for rickettsial diseases (Huang et al., 2013). Knowledge about the exposure of the OHR to typhus-causing *Rickettsia* in different environments will improve the understanding of the epidemiology of these typhi and generate baseline information that can be used for disease management and prevention. Thus, the goal of this study was to assess the exposure of rickettsial diseases in different habitats from the OHR using serological techniques.

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2. Materials and methods

2.1. Trap site and OHR collection

The Salaya suburb is located in the central region of Thailand (near Bangkok). It is a border region situated between agricultural fields and an urban area (Fig. 1). From 2015 to 2016, rodents were captured using 30 Thailand-made live traps per trap site on every trap night at approximately 10-m intervals by a 10-m transect line. Rodents were baited with dried seafood (i.e., squid and fish) or corn and jack fruit. The night traps were set up overnight and checked the next morning. The suburb rodents were captured from six trap sites over two trap nights per individual, with two seasonal traps between the wet and dry season. Six different trap sites covered three distinct rodent habitats, including (1) a human residence, with trapping occurring outside on the ground of the trap site, including a residential area and fresh market, with about a 600-m distance between sites; (2) an agriculture field, with trapping occurring in a paddy field and around fruit trees of the trap site, with a distance of 3000 m; and (3) an animal shelter, with trapping occurring around the cages or food storage areas of the trap site between the dog shelter and horse stable, with a distance of 800 m. At Faculty of Veterinary Science, Mahidol University, the captured rodents were transferred by an individual trap that was covered with a cotton bag and anesthetized by inhalation with isoflurane before measuring the main biological and morphometric parameters (cm) to identify the rodent species, including body, tail, ear, and hind foot length (mm); gender (female/male/unidentified: F/M/U); reproductive status (adult/juvenile); body weight (g); trap site; and season of collection. Blood samples were obtained from the anesthetized rodents using heart puncture. The blood was allowed to clot and was then extracted and serum collected. All collected serum was cryopreserved at −20 °C before being transferred to AFRIMS to determine rickettsiae exposure using a standard indirect immunofluorescence assay (IFA). All procedures associated with the animals in this study were approved by the Faculty of Veterinary Science –Animal Care and Use Committee (FVS–ACUC) Mahidol University, protocol no. MUVS-2015-71.

2.2. Detection of rickettsiae exposure

The specific antibody for scrub typhus in the OHR was determined using an IFA, based on a previous description by (Bozeman and Elisberg, 1963) with some modification to detect murine and tick typhus. Briefly, the samples were serially diluted twofold from 1: 50 to 1: 12,800 with phosphate-buffered saline (PBS, pH 6.8) to determine the titer of the specific antibody to *O. tsutsugamushi*, *R. typhi*, and *R. honei*. The antigens of *O. tsutsugamushi* (mixed Karp, Kato, and Gillian), *R. typhi,* and *R. honei* were prepared on glass slides with PBS and fixed with acetone. An individual dilution of the test serum was dropped onto the prepared antigen before incubating at 37 °C for 30 min and then washed with PBS and distilled water for 5 min. Fluorescein isothiocyanate (FITC) was applied to all dilutions before another incubation period and washing process. This IFA was verified using a control serum with a rat hyperimmune serum, which was produced in-house for positive control and normal rat serum for negative control. Because IFA is considered to be a serological reference test (Koh et al., 2010), and this sample was collected from nonendemic areas of rickettsiae, the presence of the specific antibody at a 1:100 or higher dilution was considered to be positive (Brown et al., 1983).

2.3. Data analysis

The number of rats was divided into each individual rickettsiae exposure to scrub, murine, and tick typhus, and the different rodent habitat in this study. The seroprevalence with 95% confidence interval (95%CI) was calculated for the individual variable association. The connection between individual rickettsial infection and compared variable distinct group was made using a χ^2 test, with variables being significant when the alpha value was less than 0.05.

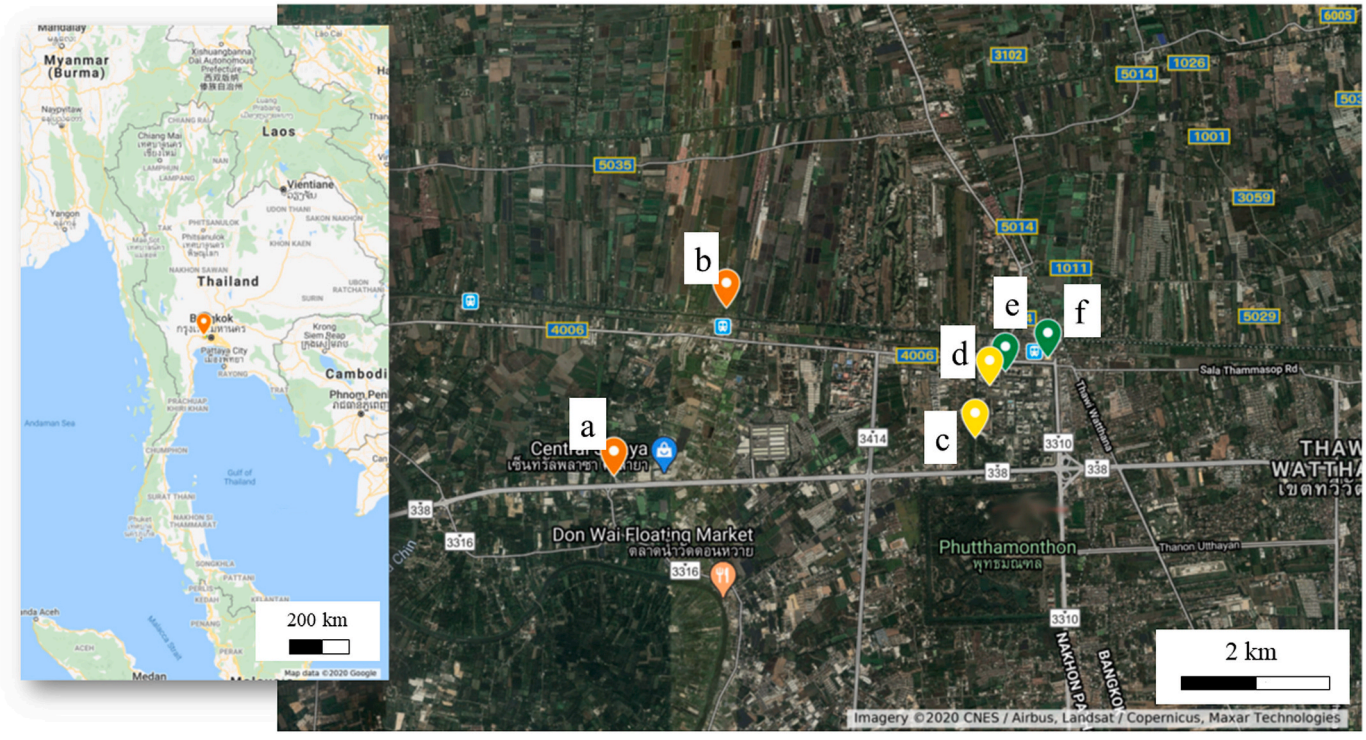

Fig. 1. The oriental house rat (*Rattus tanezumi*) trapped in a location of Salaya suburb, near Bangkok shown in a map of Thailand (scale bar; 200 km) and the satellite view of Salaya suburb (scale bar; 2 km); showing six trap site of three distinct oriental house rat habitats including I) an agriculture field (red pin): fruit tree (a) and paddy field (b); II) an animal shelter (yellow pin): dog shelter (c) and horse stable (d) and III) a human residence (green pin): residential area (e) and fresh market (f). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. Rodent trapping

A total of 237 rodents were trapped from the Salaya suburb of Bangkok (the capital city of Thailand) during 720 night traps at six trap sites (two trap sites from the individual habitats, including the animal shelter, agricultural field, and human residence) (Fig. 1.). The trap success rates (i.e., individuals per trap night from each habitat) from the animal shelter, agricultural field, and human residence were 28.33%, 32.08%, and 38.33%, respectively, and were similar ($\chi 2 = 3.72$, df = 2, $p = 0.16$) among habitats. The trapped rodents belonged to two genera and covered five species: bandicoot rat (*Bandicota indica*), Savile's bandicoot rat (*B. savilei*), polynesian rat (*Rattus exulans*), brown rat (*R. norvegicus*), and oriental house rat or OHR (*R. tanezumi*), as well as unidentified species (supplementary data). Among these rodents, the OHR was the most prevalent rodent (n = 185; 78.06% of trapped rodent) found in the Salaya suburb and was trapped from all three habitats including animal shelter (n = 65; F/M/U = 43/20/2), agricultural field (n = 67; F/M/U = 28/39/0) and human residence (n = 53; F/M/U = 36/16/1). To differentiate the rickettsial exposure among the rodent habitats, the specific antibody to the rickettsiae was detected among the OHRs.

3.2. Serological detection

A total of 185 OHRs were tested for specific antibodies against *O. tsutsugamushi*, *R. typhi*, and *R. honei* to indicate exposure to the diseases of scrub typhus, murine typhus, and tick typhus, respectively. By using an IFA, the rat serum was used to determine rickettsial exposure (Fig. 2). Exposure to murine typhus was associated with a higher antibody titer from the animal shelter and human residence than of scrub typhus and tick typhus, despite the fact that all samples from the agricultural field surprisingly lacked exposure to murine typhus. Moreover, either scrub typhus or tick typhus was found in the exposure in those rats from all three different habitats (Table 1). We found that the proportions of OHR seropositive to scrub typhus and murine typhus were significantly associated with animal shelters and human residences, respectively (scrub typhus; $\chi 2 = 13.82$, df = 2, $p = 0.001$: murine typhus; $\chi 2 = 58.61$, df = 2, $p < 0.001$). Surprisingly, there was no antibody-positive murine typhus exposure from the agricultural fields. Moreover, it was remarkable that OHR tick typhus exposure was not associated ($\chi 2 = 3.27$, df = 2, $p = 0.195$) with the habitat of the study site.

4. Discussion

Synanthropic rodent species probably transfer common pathogens to humans, particularly in a variety of environments on the rural–urban border, which is a common habitat of OHRs with high adaptation to a new geographical habitat (Blasdell et al., 2015; Heaney and Molur, 2016). To demonstrate that these OHRs are probably associated with the rickettsial pathogens in a suburb of Bangkok, Thailand, and as a preliminary study for the region, we performed a serosurvey of rickettsial diseases using IFA to explore pathogen exposure to vector-borne rickettsial diseases, including scrub typhus, murine typhus, and tick typhus. Although the serosurvey in different small mammals and humans from a scrub typhus–endemic area used different titers as the cutoff, the seropositive cutoff was equal to or greater than 1:100 according to the fourfold rise in the single serum based on the seronegative results indicating that no antibody titer was higher than 1:25 from the non-endemic area of scrub typhus, as discussed previously (Brown et al., 1983). Consequently, the seropositivity was overestimated and the disease exposure underestimated when either a higher or lower antibody titer than 1:100 dilution used to be the cutoff. Despite the pathogen sources and natural route of transmission possibly observed from the arthropod vector, this vector did not represent the rickettsial exposure in the natural host, and the individual vector showed specific rickettsiae infection, resulting the un-coverage all targeting rickettsiae. Therefore, the current study especially represents the rickettsial exposure based on serological evidence in specific high adaptive rodent species, such as OHRs. Based on the previous knowledge of transmission involving either the natural reservoir rodent or an arthropod vector, the present result of rodent habitat related to individual rickettsial diseases was conducted with the OHR exposure.

The results of the rodent trapping revealed that the OHR is the most adaptable and predominant species, as it was generally found in the Salaya suburb, covering the studied habitat. Furthermore, we found that the OHR is likely to introduce rickettsial diseases in the habitats in this study. A significant prevalence of scrub typhus was found in the animal shelter where food and bedding material were stored. This storage area was attractive to the various species of rodents, including the OHR. In addition, during this rodent assemblage the arthropod vector as well as chigger mites was possibly transferred from a natural reservoir host, in particular, either bandicoot rat or black rat (*R. rattus*) to other rodents including OHR. The relationship between bandicoot and OHR was consistent with the infectious organism introduction, as has been reported previously, by harboring the similar Hantavirus, supporting the close relationship to the exchange of pathogens, including *O. tsutsugamushi* (Johansson et al., 2010; Morand et al., 2015; Rodkvamtook et al., 2013). This finding is notable in that OHR also possibly acts as a natural host of chigger mites, similar to the bandicoot rat and black rat, even though the exposure rate of scrub typhus was 16.67%. More detailed study of ectoparasites of the OHR is needed, in terms of species diversity, to support whether the chigger mite is an accidental or natural parasite of this rat.

Interestingly, based on OHR exposure, we found that the human residential habitat was significantly associated with murine typhus. In this study, we trapped OHRs around human residences and market

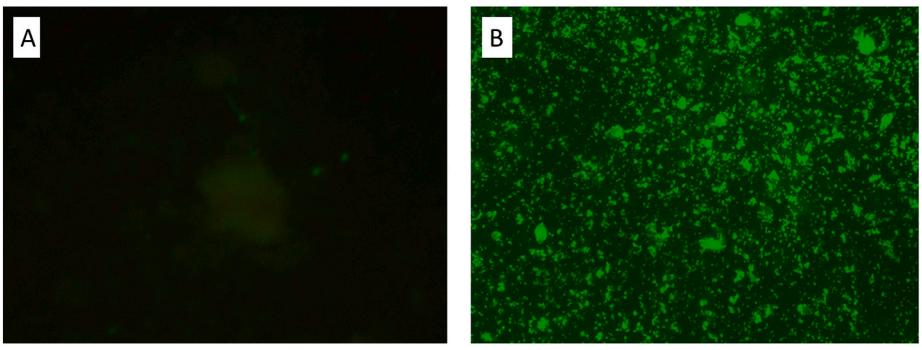

Fig. 2. Indirect immunofluorescence assay (IFA) shows the negative (A) and positive (B) reaction against *Rickettsiae* antigen with different signal of green fluorescence. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Presence of antibody titer and exposure rate to rickettsial diseases in oriental house rat from Salaya suburb, Thailand.

Reciprocal titer		Animal Shelter			Agricultural Field			Human Residence			Total (OHR)
	RE	MT	ST	TT	MT	ST	TT	MT	ST	TT	
0		43	40	51	67	54	58	18	51	38	
50		1	6	–		3	–	1	–	1	
100		–	8	7		3	–	4	2	3	
200		3	8	6		6	–	5	–	2	
400		3	1	1		–	5	11	–	3	
800		1	2	–		–	2	5	–	5	
1600		6	–	–		1	1	6	–	1	
3200		4	–	–		–	1	2	–	–	
6400		3	–	–		–	–	1	–	–	
12,800		1	–	–		–	–	–	–	–	
No. OHR			65			67			53		185
No. Positive	MT	21	–	–	0	–	–	34	–	–	55
OHR		(32.31%:			–			(64.15%:			(29.73%:
(Exposure		29.02–35.59)						62.67–65.63)			24.15–35.31)
Rate: 95%	ST	–	19	–	–	10	–	–	2	–	31
CI)			(29.23%:			(14.93%:			(3.77%:		(16.76%:
			25.68–32.78)			9.47–20.38)			0–13.10)		9.18–24.33)
	TT	–	–	14	–	–	9	–	–	14	37
				(21.54%:			(13.43%:			(26.42%:	(20.00%:
				15.87–27.21)			7.45–19.42)			22.78–30.05)	12.77–27.23)

IFA, Immunofluorescence assay; MT, Murine typhus; OHR, Oriental house rate; RE, Rickettsial exposure; ST, Scrub typhus; TT, Tick typhus.

areas, in particular outside the kitchen, where food scraps are frequently found. This food resource is possibly attractive among synanthropic species such as the brown rat, black rat, Polynesian rat, and other highly adaptive species such as the OHR. These species are abundant with rat fleas and possibly transmit the *R. typhi* together (Chareonviriyaphap et al., 2014; Morand et al., 2015). Thus, it is reasonable that the results of the current study found a significantly high prevalence of murine typhus in OHR. Nevertheless, it is surprising that no murine typhus exposure was detected in agricultural fields, either paddy fields or fruit farms, despite the assessment of 67 OHRs. The negative result found for this habitat was possibly less related to insect vectors (particularly to rat fleas), consequently indicating that this OHR lacked murine typhus exposure. This finding suggests that this agricultural field considerably minimized risk of exposure to murine typhus than other OHR habitats.

Based on the results of the pathogen exposure of the OHR from agricultural fields, a decreasing trend was found for all three rickettsial exposures, including tick typhus. However, tick typhus showed general exposure in the various habitats studied. This is likely due to an abundance of vectors of tick typhus as *Ixodes granulatus*. In comparison, *Haemaphysalis longicornis* demonstrates varying seasonal abundance during the course of a year among individual stages (larvae, nymphs, and adult stage), in particular in warm and humid weather (Zheng et al., 2012) as well the weather in the current study, resulting in the continued abundance of the transmitting vector of tick typhus. The fact that rickettsiae including *R. honei* showed transovarial and transstadial transmission in hard ticks (Kuo et al., 2011) indicates that maintaining the rickettsia within all stages in every generation causes a high efficiency and those individual stage of hard tick plays the role of the reservoir host as well as the vector at the same time. This sustainable circulation of rickettsiae in all life stages of the potential vector and reservoir host results in the transmission of *R. honei* that is generally found in OHR from all of the different habitats of this study.

In conclusion, the OHR is considered to be a general reservoir host of scrub, murine, and tick typhus because of the general exposure detected in this study. The rickettsial exposure related to the specific individual habitat is one of the potential factors. This study shows that OHR from different habitats are at risk of individual disease exposure. However, the evidence of different exposures was collected by an indirect study of synanthropic rodent from individual habitats. These habitats were usually invaded by humans for different activities. Therefore, a person working in these habitats of OHR infected with rickettsiae could be at risk of different sporadic rickettsial infections.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2020.07.015.

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