Parasites & Vectors

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Prevalence of antibodies against *Ehrlichia* spp. and *Orientia tsutsugamushi* in small mammals around harbors in Taiwan

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Abstract

Background: Tick-borne ehrlichiosis and mite-borne scrub typhus represent important emerging zoonotic rickettsial diseases. Although scrub typhus has been recognized by the Taiwanese public health system, information on ehrlichial infections is scarce in Taiwan. In this study, the risk of spread of ectoparasites on rodents through aerial and marine transportation was assessed in international and domestic harbors. Here, we report the first systematic surveillance of seroprevalence against *Ehrlichia* spp. in small mammals on the main island of Taiwan.

Methods: In total, 1648 small mammals were trapped from 8 international ports, 18 domestic fishing harbors, and 7 local public health centers around Taiwan from November 2004 to December 2008. Sera were analyzed using indirect immunofluorescence assays to detect IgG antibodies against *Ehrlichia chaffeensis* and *Orientia tsutsugamushi*. A serum titer of ≧1:80 was considered positive.

Results: Antibodies against *Ehrlichia* spp. and *O. tsutsugamushi* were detected in 3.28 % and 4.92 % of small mammals active around harbors, respectively. The seropositive rate against *Ehrlichia* was higher in northern Taiwan from 2005 to 2008. However, *O. tsutsugamushi* infections increased in southern Taiwan during this period. The serological evidence of ehrlichial and *O. tsutsugamushi* infections in all international ports were included in the study. No significant differences were found among the seropositive rates of *Ehrlichia* spp. and *O. tsutsugamushi* in small mammals trapped between international and local harbors.

Conclusions: The overall prevalence of *Ehrlichia* spp. and *O. tsutsugamushi* infections in small mammals active around harbors was 3.28 % and 4.92 %, respectively. The results provided serological evidence supporting the potential risks of transporting pathogens through air and maritime traffic. This study highlights serious issues of the emergence and spread of rickettsial diseases in Taiwan. The incidence of human ehrlichiosis requires further investigation.

Keywords: *Ehrlichia chaffeensis*, *Orientia tsutsugamushi*, Small mammals, Taiwan

Background

Rickettsial infections are caused by several genera of obligate intracellular Gram-negative bacteria with life cycles that involve vertebrate and invertebrate hosts. Different types of rickettsiosis are widely distributed worldwide, but the vector species and associated human illnesses are distinct depending on geographical locations. Rickettsioses are classically divided into the scrub typhus group, which is transmitted by mites, the spotted fever group, which is transmitted by ticks or mites, and the typhus group, which is primarily transmitted by lice or fleas. In Taiwan, notifiable rickettsial diseases include scrub typhus, epidemic typhus, and murine typhus. No epidemic typhus has been identified in Taiwan since World War II. Murine typhus, caused by *Rickettsia typhi*, is endemic to Taiwan, with approximately 20–60 cases each year. Scrub typhus group rickettsiosis is the most prevalent infection and causes 300–

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500 confirmed cases annually (http://www.cdc.gov.tw/). Scrub typhus is endemic to Asia, northern Australia, and the western Pacific regions [1, 2]. Diverse strains of *Orientia tsutsugamushi*, the etiological agent of scrub typhus, have been isolated from humans and its vectors, larval-stage trombiculid mites (chiggers) [3, 4]. Epidemiological surveys have suggested considerably high prevalence of *O. tsutsugamushi* infections in wild rodents, ranging from 69.1 % to over 90 %, on the offshore and main islands of Taiwan [5, 6]. Human infections of scrub typhus are routinely diagnosed using molecular, serological and pathogen isolation with shell-vial methods performed at the Centers for Disease Control in Taiwan (Taiwan CDC).

Ehrlichia spp., another genus of bacteria responsible for rickettsial diseases in humans and wild or domestic animals, is less recognized in Taiwan. The first human case of ehrlichiosis was documented in the United States of America in 1986. Ehrlichiosis has since been reported worldwide [7]. The common pathogens that cause human infections are *Ehrlichia chaffeensis* and *E. ewingii*. The symptoms are moderate to severe and occasionally are fatal [8, 9]. Enzootic cycles of *Ehrlichia* spp. transmitted between ticks and wild animals are widespread and have been reported in Korea, Japan, and southern China, which are in geographic proximity to Taiwan [10, 11]. Previous studies have identified *E. chaffeensis* in *Rhipicephalus haemaphysaloides* and *Ixodes granulatus* ticks and rodents in Kinmen, one of Taiwan's offshore islands [12]. Here, we report ehrlichial infections in small mammal reservoirs on the main island. The potential impact of human ehrlichiosis in Taiwan has yet to be determined.

The impact of travel on the spread of infectious diseases is of great concern. Human migration has provided a shortcut for disease dissemination. When people travel, they often carry luggage, food and goods, and they are also accompanied by, as their biological microenvironments, microbes, animals, and parasites. To examine the risks arising from transportation of pathogenic *Orientia* and *Ehrlichia* through human activities, sera were collected from small mammals captured around harbors, and the prevalence of seropositivity against *O. tsutsugamushi* and *Ehrlichia* spp. was determined by immunofluorescence assay (IFA). To the best of our knowledge, this is the first systematic surveillance of ehrlichial and *Orientia tsutsugamushi* infections in their reservoirs on the main island of Taiwan.

Methods

Study area and small mammal capture

This study was conducted as part of the surveillance of the Hanta virus in rodents in Taiwan [13]. Small mammals were captured from 33 study sites, including 8 international harbors, 18 domestic fishing harbors, and 7 local public health centers around Taiwan from November 2004 to December 2008 (Fig. 1 and Table 1). The methods for small mammal capture have been described in a previous report [14]. Briefly, approximately 20–30 traps were set up each month in areas with suspected rodent activities. Trapping at international ports took place for three days each month. Sweet potatoes, peanuts and/or sausages were used as bait to attract diverse species of small mammals. Blood was collected by cardiac puncture after the captured animals were anesthetized with Zoletil 50 (Fa. Virbac, Carros, France) and was left to stand for 1 h at room temperature. Sera were separated by centrifugation, aliquoted, and kept frozen at −20 °C for later analysis.

Ethical considerations

The study was conducted following the regulations of Article 17 of Taiwan Wildlife Conservation Act and with the permission of Taiwan Centers for Disease Control. None of the trapped mammals belonged to Protected Species. All trapping and sampling procedures were performed according to U.S. CDC guidelines for sampling small mammals for virologic testing and met International Health Regulations (IHR2005). The use of animal sera for antibody detection was approved by National Taiwan University College of Medicine and College of Public Health Institutional Animal Care and Use Committee (IACUC Approval No: 20090296).
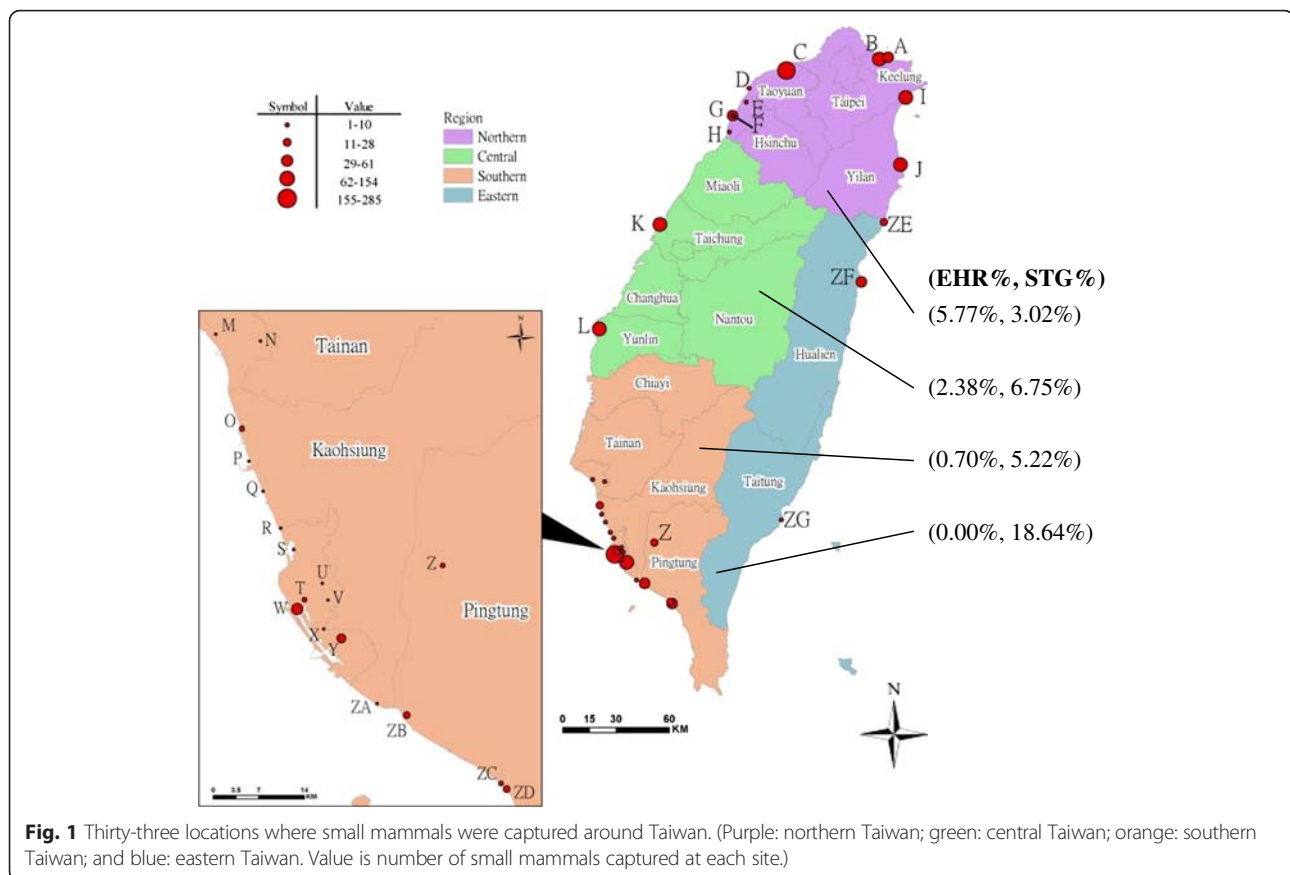
Serological analysis

Rodent serum samples were evaluated for reactivity to *E. chaffeensis* (prepared by Prof. Yu's laboratory, the University of Texas Medical Branch) and *O. tsutsugamushi* (Karp and Kato strains; prepared by Taiwan CDC) using IFA.

For detecting antibodies specific to *Ehrlichia* spp., antigen slides were prepared with *E. chaffeensis* Arkansas-infected DH82 cells. Briefly, *E. chaffeensis*-infected DH82 cells from a 150-cm^2 flask were collected when 100 % of the cells were infected. The cells were centrifuged at 200 × g for 10 min. Pellets were resuspended in 10 ml of phosphate-buffered saline (PBS) with 0.1 % bovine albumin. Sodium azide was added to a final concentration of 0.01 % and treated at 4 °C overnight to inactivate *Ehrlichia*. Ten microliters of antigen was applied to each well of 12-well slides. The slides were air dried and fixed in acetone for 5 min before immunostaining [15].

For the detection of scrub typhus antibodies, *O. tsutsugamushi* (Karp strain)-infected L929 cells on chamber slides were fixed and permeabilized with ice-cold acetone/methanol (1:1) at −20 °C for 10 min. The slides were dried and blocked with PBS containing 1 % goat serum before further immunostaining [16].

Serum samples were centrifuged at 1730 × g at 4 °C for 10 min and diluted to 1:80 using a sterile field diluent

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Fig. 1 Thirty-three locations where small mammals were captured around Taiwan. (Purple: northern Taiwan; green: central Taiwan; orange: southern Taiwan; and blue: eastern Taiwan. Value is number of small mammals captured at each site.)

(PBS containing 1.5 % bovine albumin, 100 units/mL of penicillin, 100 μg/mL of streptomycin, and 250 μg/mL of amphotericin B). A 20 μL volume of rodent serum was pipetted into each well, and the slides were incubated in a moist chamber at 37 °C for 30 min. The slides were rinsed and washed in PBS for 10 min and then rinsed again with distilled water. The slides were air-dried, and the wells were incubated with 20 μL of fluoresce in isothiocyanate (FITC)-conjugated, affinity-purified, Fc fragment-specific, goat anti-rat IgG (Jackson Immuno-Research Laboratories, West Grove, PA) diluted to 1:100 in PBS containing Evans blue counterstain (Sigma Chemical Company, St. Louis, MO). After incubation in a moist chamber at 37 °C for 30 min, the slides were rinsed and washed with PBS twice. Coverslips were mounted using mounting medium (PBS:glycerol = 3:7 v/v; pH 9.0), and the slides were examined at a magnification of 100× or 400× with a Zeiss fluorescent microscope. Sera with typical patterns of bright green fluorescence at titers of 1:80 for IgG were deemed positive [17, 18]. Positive and negative control sera were included on each slide. All slides were read independently by two individuals who were blinded to specimen identity. Disagreements were resolved by consensus after re-examining the slides.

Statistical analysis

All statistical analyses were performed using SAS v9.1.3 software (SAS Institute, Cary, NC). Fisher's exact test was used for comparisons of seroprevalence in rodents between different sampling sites. A two-tailed *p*-value of 0.05 or less was considered to be statistically significant. The trend across time was examined with the Cochran-Mantel-Haenszel Chi-squared test.

Results

Field collection of small mammals

A total of 1648 small mammals were caught from 33 study sites in Taiwan from November 2004 to December 2008, including 762, 252, 575 and 59 animals in northern, central, southern, and eastern Taiwan, respectively (Table 1). Figure 1 summarizes the numbers of trapped small mammals at the different sites. The seroprevalence of ehrlichial and *O. tsutsugamushi* infections in 4 districts of Taiwan was determined. Six species of rodents, *Rattus norvegicus* (brown rat), *Bandicota indica* (Taiwan bandicoot rat), *R. losea* (brown country rat), *Mus musculus* (house mouse), *R. tanezumi* (oriental house rat), and *Apodemus agrarius* (striped field mouse), and a species of insectivore, *Suncus murinus* (Asian house shrew),

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Table 1 List of the 33 locations around Taiwan where small mammals were captured and rates of seropositivity against *Ehrlichia* spp. and *Orientia tsutsugamushi* from November 2004 to December 2008

Geographic	Location ([a])	Total no. trapped mammals (No. sero-positive to EHR, no. sero-positive to STG)					
		2004	2005	2006	2007	2008	Total (2004 ~ 2008)
Northern		0	140 (2,2)	186 (16,6)	134 (12,4)	302 (14,11)	762 (44,23)
	A. Badouzih Fishing Harbor (KL)	0	33	25	0	0	58
	B. Keelung Harbor (KL)[b]	0	44 (2,2)	38 (3,2)	22 (0,1)	30 (1,2)	134 (6,7)
	C. Taoyuan Airport (TY)[b]	0	30	71 (8,2)	80 (12,1)	64 (2,1)	245 (22,4)
	D. Yongan Fishing Harbor (TY)	0	0	0	0	6	6
	E. Potou Fishing Harbor (HC)	0	0	1	0	0	1
	F. Nangliao Fishing Harbor (HC)	0	0	9	0	0	9
	G. Hsinchu Fishing Harbor (HC)	0	0	0	0	61 (2,2)	61 (2,2)
	H. Haishan Fishing Harbor (HC)	0	0	0	0	5	5
	I. Dashi Fishing Harbor (YL)	0	0	5	0	84 (6,3)	89 (6,3)
	J. Suao Harbor (YL)[b]	0	33	37 (5,2)	32 (0,2)	52 (3,3)	154 (8,7)
Central		1	100 (2,6)	66 (4,9)	40	45 (0,2)	252 (6,17)
	K. Taichung Wuchi Harbor (TC)[b]	0	27 (0,1)	34 (1,2)	22 (0,0)	29 (0,2)	112 (1,5)
	L. Mailiao Harbor (YL)[b]	1	73 (2,5)	32 (3,7)	18 (0,0)	16 (0,0)	140 (5,12)
Southern		10	193 (0,4)	130 (3,4)	85 (0,10)	157 (1,12)	575 (4,30)
	M. Anping Fishing Harbor (TN)	0	0	2	0	0	2
	N. East District, Tainan city (TN)	0	0	2	0	0	2
	O. Hsinda Fishing Harbor (KH)	0	0	16	3	9	28
	P. Hsingang Fishing Harbor (KH)	0	0	1	0	0	1
	Q. Mito Fishing Harbor (KH)	0	0	0	0	4	4
	R. Uhliao Fishing Harbor (KH)	0	0	2	3	5	10
	S. Zhoying Military Area (KH)	0	0	7	0	0	7
	T. Qianzhen District, Kaohsiung City (KH)	0	0	0	0	15	15
	U. Sanmin District, Kaohsiung City (KH)	0	0	0	0	1	1
	V. Yanchao District, Kaohsiung City (KH)	0	0	4	0	0	4
	W. Kaohsiung International Harbor (KH)[b]	8	65 (0,2)	51 (0,1)	67 (0,7)	94 (1,9)	285 (1,19)
	X. Chungyun Fishing Harbor (KH)	0	0	3	0	0	3
	Y. Kaohsiung International Airport (KH)[b]	2	41	30 (2,3)	12 (0,3)	11 (0,0)	96 (2,6)
	Z. Yanpu Fishing Harbor (PT)	0	0	0	0	16 (0,3)	16 (0,3)
	ZA. Linyuan Fishing Harbor (KH)	0	0	0	0	2	2
	ZB. Donggang Fishing Harbor (PT)	0	32	6	0	0	38
	ZC. Shueidiliao Fishing Harbor (PT)	0	16	0	0	0	16
	ZD. Fangliao Fishing Harbor (PT)	0	39 (0,2)	6 (1,0)	0	0	45 (1,2)
Eastern		0	6 (0,1)	9 (0,1)	19 (0,2)	25 (0,7)	59 (0,11)
	ZE. Hoping Fishing Harbor (HL)	0	0	0	4 (0,2)	16 (0,6)	20 (0,8)
	ZF. Hualien Harbor (HL)[b]	0	6 (0,1)	6 (0,1)	15	9 (0,1)	36 (0,3)
	ZG. Fugang Fishing Harbor (TT)	0	0	3	0	0	3
Total		11 (0,0)	439 (4,13)	391 (23,20)	278 (12,16)	529 (15,32)	1648 (54,81)

KL Keelung, *TY* Taoyuan, *HC* Hsinhu, *TC* Taichung, *YL* Yunlin, *TN* Tainan, *KH* Kaohsiung, *PT* Pingtung, *TT* Taitung, *HL* Hualien, *YL* Yilan

[a]Name of County or City in Taiwan

[b]International Harbor

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were identified in this study. The numbers of each species captured and the animals' genders are outlined in Table 2. *Rattus norvegicus*, *S. murinus*, and *B. indica* were the predominant species, accounting for 1102, 284 and 182 of the trapped small mammals, respectively.

Serological analyses of captured animals

Antibodies against *Ehrlichia* spp. and *O. tsutsugamushi* were found in 3.28 % (54/1648) and 4.92 % (81/1648) of the captured small mammals on the main island of Taiwan, respectively (Table 2). The prevalence was significantly different between ehrlichial (2.99 %; 33/1102) and *O. tsutsugamushi* infections (6.26 %; 69/1102) in *R. norvegicus* ($p < 0.01$). The gender of the animals captured appeared to have no influence on the prevalence of both rickettsial infections. Among the rodents, *B. indica* displayed the highest positive rate of 10.44 % (19/182) against *Ehrlichia* spp., and *Ratus tanezumi*

Table 2 Species and genders of the captured small mammals and the prevalence of antibodies against *Ehrlichia* spp. and *Orientia tsutsugamushi* in small mammals in Taiwan from November 2004 to December 2008

| Species | Sex | Total | EHR | | STG | | p value |
			Positive	%	Positive	%	
Rattus norvegicus	M	602	17	2.82	36	5.98	0.0293
	F	489	13	2.66	31	6.34	0.0244
	U	11	3	27.27	2	18.18	1.0000
	total	1102	33	2.99	69	6.26	0.0023
Suncus murinus	M	102	0	0.00	4	3.92	0.1213
	F	179	0	0.00	0	0.00	-
	U	3	0	0.00	0	0.00	-
	total	284	0	0.00	4	1.41	0.1237
Bandicota indica	M	130	13	10.00	1	0.77	0.0014
	F	52	6	11.54	1	1.92	0.1123
	total	182	19	10.44	2	1.10	0.1886
Rattus losea	M	35	2	5.71	1	2.86	1.0000
	F	15	0	0.00	2	13.33	0.4828
	total	50	2	4.00	3	6.00	1.0000
Mus musculus	M	6	0	0.00	0	0.00	-
	F	10	0	0.00	0	0.00	-
	total	16	0	0.00	0	0.00	-
Rattus tanezumi	M	5	0	0.00	0	0.00	-
	F	8	0	0.00	3	37.50	0.2000
	total	13	0	0.00	3	23.08	0.2200
Apodemus agrarius	M	1	0	0.00	0	0.00	-
	F	0	0	–	0	–	-
	total	1	0	0.00	0	0.00	-
Total		1648	54	3.28	81	4.92	

M male, *F* female, *U* unknown

exhibited the highest infection rate against *O. tsutsugamushi* (23.08 %; 3/13).

Seroprevalence in small mammals captured in different geographic locations in Taiwan

The serological survey revealed that antibodies specific to *Ehrlichia* spp. were most frequently found in small mammals active around the ports in northern Taiwan (5.77 %; 44/762), followed by the central (2.38 %; 6/252) and southern (0.70 %; 4/575) districts. The percentage of samples positive for antibodies specific to *Ehrlichia* spp. was highest in Taoyuan Airport (8.98 %; 22/245). Furthermore, in agreement with the incidence in human cases, we detected a high prevalence of antibodies against *O. tsutsugamushi* at the Hoping Fishing Harbor (40 %; 8/20), which is located in eastern Taiwan (Table 1 and Fig. 1).

In this study, 1202 and 446 small mammals were trapped at the international and domestic harbors (including local public health centers), respectively (Table 3). The prevalence of antibodies specific to *O. tsutsugamushi* was slightly higher than the prevalence of antibodies specific to *Ehrlichia* spp. Moreover, a higher but not significantly greater number of animals expressed antibodies against the rickettsial diseases around the international ports. Most importantly, serological evidence of infections was detected in all international harbors (Table 1).

Seroprevalence in small mammals captured at different time points

The seroprevalence of *O. tsutsugamushi* infections in small mammals increased in the harbors of southern Taiwan during the year of 2004 and 2008 ($p < 0.01$) (Table 4). Antibodies against *Ehrlichia* spp. and *O. tsutsugamushi* were detected year-round (Fig. 2). In trapped small mammals, the seropositive rates were highest in November and December for *Ehrlichia* spp., whereas the positive rates were higher in January and October for *O. tsutsugamushi*.

Co-infection with different pathogens in captured animals

Of the 1648 small mammals tested in the study, 5 (0.30 %) were seropositive against both *Ehrlichia* spp. and *O. tsutsugamushi*, including one *R. norvegicus* in Keelung Harbor, 2 *R. norvegicus* in Mailiao Harbor, one *M.*

Table 3 Comparison of rates of seropositivity against *Ehrlichia* spp. (EHR), and *Orientia tsutsugamushi* (STG) infections between international harbors and local harbors in Taiwan

| Locations | Total | EHR | | STG | |
		Positive	%	Positive	%
International Harbor	1202	45	3.74	63	5.24
Local Harbor	446	9	2.02	18	4.04
Total	1648	54	3.28	81	4.92

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musculus in Kaohsiung International Airport, and one *R. norvegicus* in Suao Harbor.
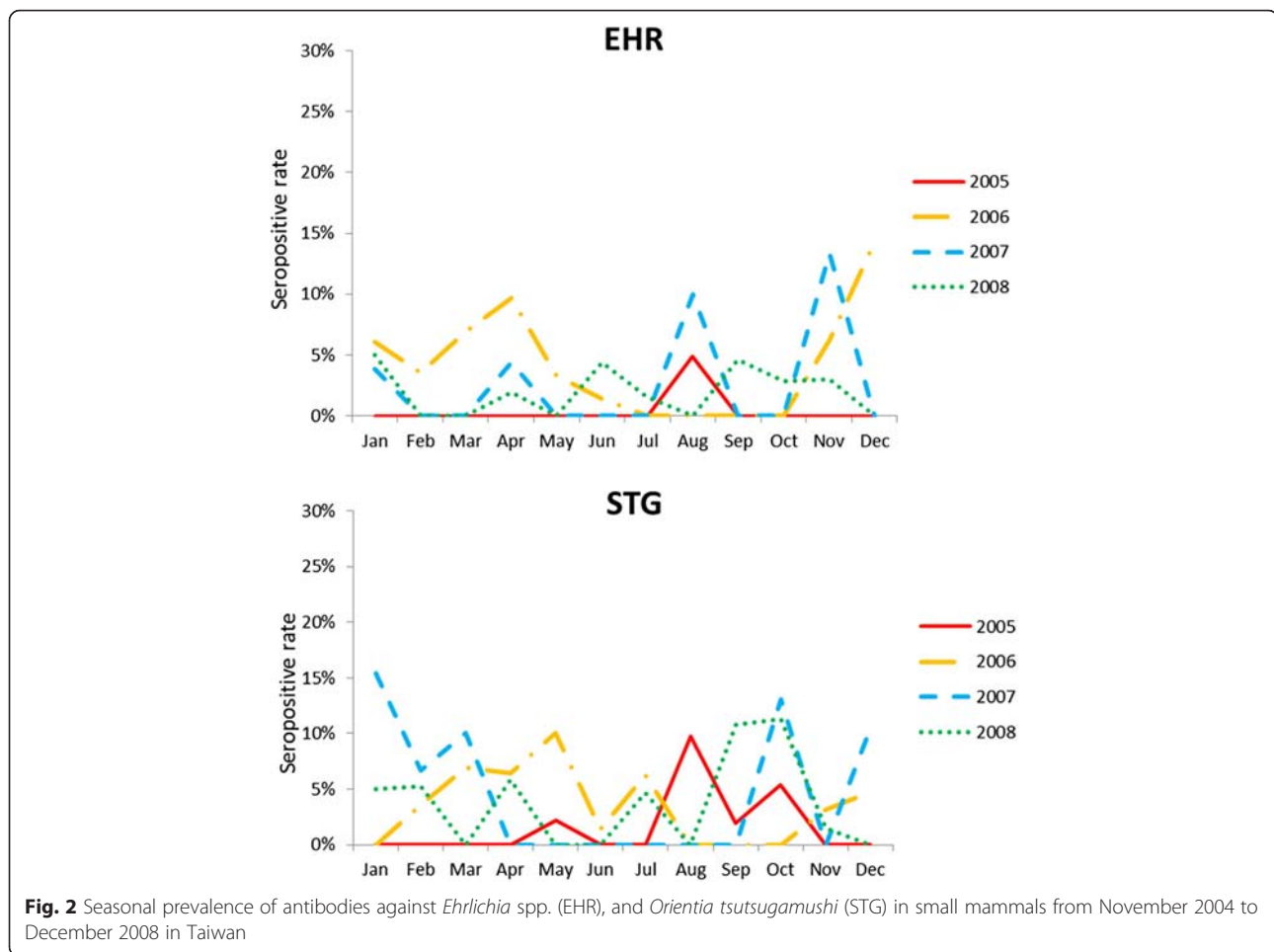
Discussion

In the study, the prevalence of *Ehrlichia* spp. and *O. tsutsugamishi* infections in captured animals was 3.28 % (54/1648) and 4.92 % (81/1648), respectively. Most of the seropositive animals were located in northern Taiwan. Serological evidence of infections were present in all international ports. The antibodies were detected in trapped small mammals year-round with an increasing trend in *O. tsutsugamushi* infections in southern Taiwan from 2004 to 2007. Moreover, this is the first report regarding the prevalence of *Ehrlichia* spp. infection in small mammals on the main island of Taiwan.

Ehrlichiosis is an important emerging zoonotic disease. Serological studies and molecular detections have associated *E. chaffeensis* with white-tailed deer, goats, red foxes, coyotes, raccoons, cattle, and Reeves's muntjacs [7, 19–25]. These large wild animals are less common in Taiwan. Conversely, shrews and rodents are more abundant and often serve as reservoirs for many communicable diseases transmitted by their ectoparasites. Previous studies have discovered *E. chaffeensis* DNA in 16 of 108 (14.8 %) spleen or liver samples from shrews and rodents on one of Taiwan's offshore islands, Kinmen, by polymerase chain reaction (PCR) and sequencing [14]. Our results further showed that 3.28 % (54/1648) of captured animals were seropositive against ehrlichial infections, and more infected animals were found in the north and central parts of the main island of Taiwan. During this study, ectoparasites collected from murine-like animals included two fleas: *X. cheopis* and *Nosopsyllus nicanus*, three ticks: *Ixodes granulatus*, *Haemaphysalis bandicota,* and *Rhipicephalus haemaphysaloides*, three mites: *Laelaps nuttalli*, *L. sedlaceki*, and *L. echidninus*, and lice. Among these ectoparasites, 45.04 % were *X. cheopis*, and 29.25 % were *H. bandicota*. In terms of the ectoparasites, *X. cheopis* was the most common species. *X. cheopis* carries *R. typhi* and serves as a major vector to cause human murine typhus. *Haemaphysalis bandicota*, the second common ectoparasite, involves in disease transmission among rats. However, *H. bandicota* has never been found to be associated with any vector-borne communicable diseases in humans [26]. Information on trombiculid mites was limited in this study, but these mites may be associated with small mammal species. It is worth noting that serological cross-activities have been addressed among *Ehrlichia* spp., such as *E. chaffeensis*, *E. ewingii*, *E. canis* and *E. ruminantium* [27–30]. The enzootic cycle of *E. canis* has been documented in dogs in Taiwan [31, 32]. The positive reactions observed by IFA in the current study therefore may have been confounded by infections with other *Ehrlichia* species. In North America, the seroprevalence of *E. canis*, *E. chaffeensis* and *E. ewingii* in dogs was 0.8 %, 2.8 %, and 5.1 %, respectively [33]. In China, the overall seroprevalence of *E. chaffeensis* was 9.8 % in rural residents and 2.4 % in urban residents [34]. Nevertheless, our results suggested that ehrlichial infections are prevalent on the island. The implications for human ehrlichiosis should be scrutinized and re-evaluated because it is not a commonly observed disease in Taiwan.

Table 4 Trends of rates of seropositivity of antibodies against *Ehrlichia* spp. (EHR) and *Orientia tsutsugamushi* (STG) in small mammals in northern, central, southern, and eastern Taiwan from November 2004 to December 2008

Variable	2004 (N = 11)		2005 (N = 439)		2006 (N = 391)		2007 (N = 278)		2008 (N = 529)		*p* value	(trend) *p* value
	N	%	N	%	N	%	N	%	N	%		
EHR												
Northern	0	0	2	0.46	16	2.81	12	4.32	14	0.95	0.0054	0.8555
Central	0	0	2	0.46	4	1.02	0	0.00	0	0.00	0.1908	0.3183
Southern	0	0	0	0.00	3	0.77	0	0.00	1	0.19	0.0845	0.8228
Eastern	0	0	0	0.00	0	0.00	0	0.00	0	0.00	-	-
Total	0	0	4	0.91	23	5.88	12	4.32	15	2.84	0.0003	0.3306
p value			0.127		0.115		0.00007375		0.040			
STG												
Northern	0	0	2	0.46	6	1.02	4	0.72	11	0.95	0.9432	0.6543
Central	0	0	6	1.37	9	2.30	0	0.00	2	0.38	0.0440	0.3680
Southern	0	0	4	0.91	4	0.77	10	3.60	12	2.27	0.0016	0.0023
Eastern	0	0	1	0.23	1	0.77	2	1.44	7	2.46	0.7194	0.2352
Total	0	0	13	2.96	20	4.86	16	5.76	32	6.05	0.1183	0.0264
p value			0.245		0.005		0.008		0.167			

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Fig. 2 Seasonal prevalence of antibodies against *Ehrlichia* spp. (EHR), and *Orientia tsutsugamushi* (STG) in small mammals from November 2004 to December 2008 in Taiwan

Several studies have evaluated the prevalence of scrub typhus infections in animal reservoirs. A seropositive rate of over 90 % has been reported in rodents in Kinmen [5]. A prevalence of 70 % has been observed in small mammals captured in abandoned agricultural fields in central Hualien County in eastern Taiwan [35]. Our results showed that 4.92 % of the small mammals caught around the harbors expressed antibodies against *O. tsutsugamushi*. The prevalence was relatively low compared with those in previous reports and may reflect the different ecosystems of our sampling areas. Different rodent hosts affect the seropositive rates of rickettsial infections [5, 12, 13]. Our study focused on small mammals active around harbors, which have, to a certain degree, much more human activities than present in the study sites selected in other studies. Furthermore, the rodent species in these harbors differed from those on small islets or in traditional agricultural environments. Indeed, the dominant species among captured animals varies in the literature. *R. norvegicus* was the dominant rodent species in this study, whereas *R. losea* and *Apodemus agrarius* are abundant in Kinmen and Hualien, respectively [5, 35].

The distributions of the rodent species were similar among harbors in Taiwan, with the exceptions of Suao Harbor and Taoyuan Airport. On the whole, *R. norvegicus* was the most frequent species followed by *S. murinus* and *B. indica*. However, the Taiwan bandicoot rat and brown country rat were the primary rodents captured in Taoyuan Airport. This may be related to the trap sites and the behaviors of *B. indica*, which are usually active in non-residential areas. *Haemaphysalis bandicota* was also primarily found at Taoyuan International Airport [26]. For Su-ao Harbor, the only species captured were *R. norvegicus* and *M. musculus* (*n* = 1).

Together, the geographic distribution, activities of the vectors and reservoirs, and human activities determine the prevalence of rickettsial disease infections. In this study, *B. indica* appeared to be most influenced by *E. chaffeensis* (10.44 %), and *R. tanezumi* had the highest rate of seropositivity against *O. tsutsugamushi* (23.08 %). Although seropositive rates were highest in November and December for *Ehrlichia* spp., whereas the positive rates were higher in January and October for *O. tsutsugamushi*. The numbers of captured animals did not

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differ significantly between seasons, and the antibodies were detected year-round. In general, our results revealed that small mammals, which are common in residences, were infected by *Ehrlichia* and *O. tsutsugamushi*. The frequent association between these mammals and human activities may increase the risk of rickettsial diseases.

Our results indicated that the rates of seropositivity against *O. tsutsugamushi* were higher in the eastern and central parts of Taiwan, which correlated with the high incidence of human scrub typhus in these regions [3]. Multiple environmental factors and vector species have been shown to affect the prevalence of vector-borne infectious pathogens in animal hosts [36–38]. A recent article has also reported a positive correlation between the rate of seropositivity in rodents and the incidence of human scrub typhus; however, the major captured animal in that study, *S. murinus*, was not tested due to a lack of antiserum [38]. FITC-conjugated goat anti-rat antibodies were used to detect antibodies from captured small mammals in our study. Similar strategies have been applied in other experiments when no antiserum was commercially available [39, 40]. Although we cannot guarantee that anti-rat secondary antibodies react with rodent and shrew sera equally, the shrew was a minor species among all trapped small mammals (17.23 %). Zero and 4 (1.41 %) shrews tested positive for antibodies against *E. chaffeensis* and *O. tsutsugamushi*, respectively.

Travel and trade have provided a source for disease dissemination throughout recorded history. Plague represents one of the most striking examples of this phenomenon. Human population movement has been crucial to the dispersal of *Yersinia pestis* and rodents and their fleas to new territories [41]. Moreover, international trade of pet reptiles and amphibians has been shown to have a potential to cause emerging infections of *Rickettsia* and *Ehrlichia* spp. in Japan [42]. In our study, animals were found to harbor antibodies against *Ehrlichia* and *O. tsutsugamushi* around ports and harbors. The prevalence of seropositivity was slightly higher in the samples collected near international harbors; however, no significant differences were observed. Nevertheless, serological evidence of infections was detected in all international harbors studied. These findings highlight the risk of spreading pathogenic *Ehrlichia* and *Orientia* associated with ectoparasites on rodents through aerial and marine transportation.

Conclusions

Our study provided serological evidence of *Ehrlichia* spp. infection in small mammals on the main island of Taiwan. The implications for human ehrlichiosis should be re-evaluated because it is not a commonly observed disease in Taiwan. Small mammals can serve as useful indicators and/or sentinels for epidemiological surveillance, disease control and prevention of vector-borne infectious diseases. The prevalence of infected animals captured around the harbors should raise awareness of the potential risks of disseminating pathogens by domestic and international aerial and marine transport.

Authors' contributions
KHT and PYS participated in the design of the study and drafted the manuscript. SFC was involved in sample and antigen preparation and provided guidance for IFA. KHT and WJC performed IFAs. WLS and THW performed the statistical analyses and geographic mapping. XJY assisted in preparing the ehrlichial antigen. KHT, TYY, HCW, WJW and PYS reviewed the data and the manuscript. All authors critically reviewed and approved the final manuscript.

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