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Identification of potential hosts and vectors of scrub typhus and tick-borne spotted fever group rickettsiae in eastern Taiwan

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Abstract. Scrub typhus and tick-borne spotted fever group (SFG) rickettsioses are transmitted by chiggers (larval trombiculid mites) and hard ticks, respectively. We assessed exposure to these disease vectors by extensively sampling both chiggers and ticks and their small mammal hosts in eastern Taiwan during 2007 and 2008. The striped field mouse *Apodemus agrarius* Pallas (Rodentia: Muridae) was the most common of the small mammals (36.1% of 1393 captures) and presented the highest rate of infestation with both chiggers (47.8% of 110 760) and ticks (78.1% of 1431). *Leptotrombidium imphalum* Vercammen-Grandjean & Langston (Trombidiformes: Trombiculidae) and immature *Rhipicephalus haemaphysaloides* Supino (Ixodida: Ixodidae) were the most abundant chiggers (84.5%) and ticks (>99%) identified, respectively. Immunofluorescent antibody assay revealed high seropositive rates of rodents against *Orientia tsutsugamushi* Hyashi (Rickettsiales: Rickettsiaceae), the aetiological agent of scrub typhus (70.0% of 437 rodents), and tick-borne SFG rickettsiae (91.9% of 418 rodents). The current study represents a first step towards elucidating the potential hosts and vectors in the enzootic transmission of *O. tsutsugamushi* and tick-borne SFG rickettsiae in Taiwan. Further studies should focus on characterizing pathogens in *L. imphalum* and *R. haemaphysaloides*, as well as the proclivity of both vectors to humans. Uncovering the main hosts of adult ticks is also critical for the prevention of SFG rickettsial infections.

Key words. *Orientia tsutsugamushi*, hosts, scrub typhus, spotted fever, vectors, Taiwan.

Introduction

Scrub typhus is an acute human infectious disease prevalent in the western Pacific region, southern Asia and northeastern Australia. Transmitted by larval trombiculid mites (i.e. chiggers) harbouring the rickettsia *Orientia tsutsugamushi* Hyashi, this disease infects about one million people annually and one billion people are estimated to be at risk (Kawamura *et al*., 1995; Rosenberg, 1997). The lifecycle of trombiculid mites includes seven stages [egg, deutovum, larva (chigger), protonymph, deutonymph, tritonymph, adult], but only in the chigger stage is the mite parasitic (Kawamura *et al*., 1995). *Leptotrombidium* chiggers are the primary vectors of scrub typhus and murine rodents, especially *Rattus* species (Rodentia: Muridae), are their predominant hosts in regions in which scrub typhus is endemic (Traub & Wisseman, 1974; Kawamura *et al*., 1995). Humans are accidental hosts and become infected with scrub typhus when they are bitten by chiggers infected with *O. tsutsugamushi*. Trombiculid mites are the only reservoirs of *O. tsutsugamushi*, which can be transmitted transstadially (from larva to nymph to adult) and transovarially (from the female to next generations); vertebrate hosts provide

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chiggers with food resources, but are of little importance in the transmission of *O. tsutsugamushi* (Kawamura *et al*., 1995).

Scrub typhus has historically been prevalent mainly on small islands around Taiwan (Lee *et al*., 2006) and extensive studies on chiggers and their hosts have focused on offshore islets, including the Penghu (Pescadores) Islands (Cooper *et al*., 1964; Lien *et al*., 1967; Olson *et al*., 1978, 1982) and Kin-men Islands (Wang *et al*., 2004). In recent decades, however, this disease has emerged on the main island of Taiwan and is currently the most important rickettsial disease in the country (Tsai *et al*., 2008a). Nonetheless, the main island has featured only in preliminary studies (Hatori, 1919; Gale *et al*., 1974; Wang, 1988, 2004; Lin, 1999), although recent increases in the incidence of scrub typhus in eastern Taiwan call for further efforts. Moreover, animal communities on islands often represent depauperate subsets of mainland faunas (Courchamp *et al*., 2003). Consequently, the chiggers and hosts in Taiwan may differ from those on surrounding islets.

Spotted fever group (SFG) rickettsioses are vector-borne zoonoses caused by rickettsiae belonging to the genus *Rickettsia* (Rickettsiales: Rickettsiaceae), most of which are transmitted by hard ticks (Ixodida: Ixodidae), although some species are transmitted by mites or fleas (Raoult & Roux, 1997). Vertebrates, especially mammals, are hosts of hard ticks (Parola & Raoult, 2001; Smith *et al*., 2008). Spotted fever group rickettsiae can be transmitted trans-stadially and transovarially in ticks and thus ticks can serve as both vectors and reservoirs of tick-borne SFG rickettsiae; it is less clear whether vertebrate hosts also serve as reservoirs of these pathogens (Raoult & Roux, 1997).

Spotted fever antibodies occur in residents of southern Taiwan, although not frequently [seropositivity rates: 3.4–4.4% (Takada *et al*., 1993)]. Spotted fever antibodies have also been reported in rodents in the Kin-men Islands (66.4% positive) and northern Taiwan (42.9% positive) (Chen *et al*., 1997). Moderate rates of seropositivity have been documented in rodents and shrews in both the Ma-tsu [*Rickettsia rickettsii* (Wolbach): 50.7%; *Rickettsia conorii* (Brumpt): 37.3%] and Kin-men (*R. rickettsii*: 72.7%; *R. conorii*: 59.0%) Islands (Wu, 2006), although the aetiological agents may be other *Rickettsia* spp. cross-reacting with commercially available *R. rickettsii* and *R. conorii* antigens. Recently, molecular techniques have facilitated the identification of some novel SFG rickettsiae in ticks, mites and fleas in Taiwan (Tsui *et al*., 2007; Tsai *et al*., 2008a, 2009), as well as flea-borne *Rickettsii felis* Higgins, Radulovic, Schriefer & Azad in humans (Tsai *et al*., 2008b).

Although no human illness caused by tick-borne SFG rickettsiae has yet been reported in Taiwan (Tsai *et al*., 2008b), the fact that many tick-borne SFG rickettsioses have emerged in recent years (Parola *et al*., 2005) indicates that a good knowledge of potential tick vectors and their vertebrate hosts in the natural foci of SFG rickettsiae will be valuable for assessing human risk for spotted fever infection. However, past studies on ticks and hosts in Taiwan focused on ad hoc collections of ticks in different parts of the country (Shih, 2002; Tsai, 2002). Extensive collections of ticks have been made in the Ma-tsu and Kin-men Islands, although the species of ticks recovered were not reported (Chang, 2002; Wu, 2006). Only Wang (2005) briefly documented the main tick and host species in the Kin-men Islands.

Chiggers, ticks and their small mammal hosts were sampled in eastern Taiwan and seroprevalences of *O. tsutsugamushi* and tick-borne SFG rickettsiae of hosts were assessed. In the current study, hosts were defined solely as providers of food resources and no implications of their status as disease reservoirs were made. Our samples were extensive and systematic, and provide documentation of the temporal patterns of abundance of both chiggers and ticks in Taiwan. The current paper reports on these patterns and thus provides the first insights on potential vectors and hosts for SFG rickettsiae in Taiwan, and on *O. tsutsugamushi* on the main island of Taiwan.

Materials and methods

Study area

The current study was conducted in abandoned agricultural fields in the lowlands of Shou-feng and Fong-lin in central Hua-lien County in eastern Taiwan, where agriculture dominates land use and villages are interspersed among fields. Although Hua-lien is one of Taiwan's least populated counties, it reported the country's second highest number of human cases of scrub typhus between 1998 and 2007 (totalling 525 cases), exceeded only by the Kin-men Islands (558 cases) (Centres for Disease Control, 2008). Scrub typhus has been endemic in Hua-lien for at least 95 years (Hatori, 1919). Spotted fever group rickettsiae have recently been documented in Hua-lien, where a novel strain (*Rickettsia* sp. TwKM01) was recovered from the hard tick *Rhipicephalus haemaphysaloides* Supino (Tsui *et al*., 2007).

Small mammal trapping and collection of ticks and chiggers

Abandoned agricultural plots were surveyed from January to March 2007 and from August 2007 to March 2008 (except November 2007). From January to March 2007, 14 plots were sampled. All sampling deployed two parallel transect lines containing 10 Sherman traps (26.5 × 10.0 × 8.5 cm) placed at 10-m intervals and two meshed live traps (27.0 × 16.0 × 13.0 cm) placed at 50-m intervals. Meshed traps were used to target less abundant, but larger greater bandicoot rats [*Bandicota indica* (Bechstein) (Rodentia: Muridae)]. Adjacent transect lines were separated by 10 m. After August 2007, trapping effort was increased, sampling 35 additional fields with three parallel transect lines containing 10 Sherman traps at 10-m intervals, and three meshed live traps at 30-m intervals in each plot. Adjacent transect lines were separated by 10 m. Traps were baited with sweet potato covered with peanut butter in the evening and checked for captures early in the morning. All sites were surveyed for three consecutive nights.

Trapped small mammals (rodents and shrews) were transferred to clean nylon mesh bags; bags were carefully examined to ensure that no ectoparasites remained from earlier captures. Rodents were anaesthetized with Zoletil® 50 (Virbac SA, Carros, France) and examined for ectoparasites by combing

their fur thoroughly. We also recorded gender, reproductive status, body weight (g) and length of body, tail, ear, and hind-foot (mm). Chiggers were recovered during all sampling periods; ticks were collected during most periods (except January and February 2007). Ticks were carefully collected with tweezers and were then directly preserved in 70% ethanol. Skin with attached chiggers was detached with minimum injury to the host animal and preserved in vials; chiggers released themselves from the skin and were transferred to 70% ethanol after 2 days. Ticks and chiggers recovered from animals were counted individually. About 0.2 mL of blood, collected from the submandibular area or from the saphenous vein, was centrifuged and sera stored at −70 °C for later determination. Rodents were marked with fur clips and released at least 5 km away from the study areas. Shrews were initially screened for ectoparasites. Shrews infested with chiggers were killed with an overdose of Zoletil® 50 and blood was collected via heart puncture. Those without ectoparasites or infested only with ticks were marked with fur clips and released outside the study area without collecting blood. All ticks were recovered. The same external measurements of shrews and rodents were recorded. All procedures were approved by the University of California Davis Animal Use and Care Administrative Advisory Committee and met guidelines recommended by the American Society of Mammalogists (Gannon *et al*., 2007).

Tick and chigger identification

At least one-fifth of chiggers were randomly selected from each host individual for species identification. Chiggers were soaked in deionized water two or three times (30 min each) and then slide-mounted in Berlese fluid (Asco Laboratories Ltd, Manchester, U.K.). Chiggers were examined under a light microscope and identified according to published keys (Wang & Yu, 1992; Li *et al*., 1997). Ticks were examined under a dissecting microscope and identified using published keys (Teng & Jiang, 1991). Unidentified ticks were confirmed by comparing 12S rDNA sequences with those of known species following Beati & Keirans (2001).

Immunofluorescent antibody assay

Rodents were assayed for chigger-borne scrub typhus (*O. tsutsugamushi*) and tick-borne SFG rickettsiae. Each serum sample was diluted 1 : 40 in phosphate-buffered saline (PBS) and applied to slides coated with antigens (*O. tsutsugamushi*: Gu-Yuan Biotech Ltd, Taipei, Taiwan; SFG rickettsiae: Focus Technologies, Inc., Cypress, CA, U.S.A.). Because *R. conorii* antigens cross-react poorly or not at all with non-tick-borne *Rickettsia* spp. (i.e. flea-borne *Rickettsia typhi* Wolbach & Todd, *R. felis*; louse-borne *Rickettsia prowazekii* da Rocha-Lima; mite-borne *Rickettsia akari* Huebner, Jellison & Pomerantz), and typhus group rickettsiae (*R. typhi*, *R. prowazekii*, *Rickettsia canada* McKiel, Bell & Lackman) (Fang & Raoult, 2003), we used commercial slides coated with *R. conorii* antigens (product code IF0104; Focus Technologies, Inc.) to screen for tick-borne SFG rickettsiae. Slides were then put in a humid chamber at 37 °C for 30 min, soaked in PBS for 5 min, washed with deionized water, allowed to dry and pipetted with fluorescein isothiocyanate-goat anti-mouse IgG + A + M(H + L) (Zymed Laboratories, Inc., San Francisco, CA, U.S.A.) diluted 1 : 40 in PBS. Slides were incubated again in a humid chamber at 37 °C for 30 min, washed with PBS, allowed to dry and fitted with coverslips. We used sera from wild rodents with polymerase chain reaction-confirmed *O. tsutsugamushi* or SFG rickettsiae infections in their livers, spleens and kidneys as positive controls, and sera from these rodents without infections as negative controls. Assays were considered positive when bright green fluorescence matched those of positive controls under a fluorescence microscope (Leica DM2500 Fluorescence Microscope; excitation wavelength: 465–495 nm; emission wavelength: 515–555 nm; Leica Microsystems GmbH, Wetzlar, Germany). *Orientia tsutsugamushi* antigen slides allowed simultaneous screening of three strains (Kato, Karp and Gilliam). Serum samples were scored negative for *O. tsutsugamushi* when all three strains were negative; if any strains yielded positive results, the sample was recorded as positive.

Human cases of scrub typhus in Hua-lien County

Reporting of scrub typhus is mandatory in Taiwan and the Database of Infectious Diseases (administrated by the Centres for Disease Control, 2008) includes the date, location and related information on the patient for each human case. Records of confirmed human cases of scrub typhus in Hua-lien County for the period January 2007 to April 2008 were retrieved. Because the latency period for scrub typhus is 9–12 days (Lee *et al*., 2006), the date of infection was estimated as 10 days prior to the onset of symptoms.

Statistical analysis

Relationships between mean parasite loads and chigger or tick prevalence were assessed across host species, as was the relationship between seasonal scrub typhus human cases and mean chigger loads on the striped field mouse *Apodemus agrarius* Pallas, using parametric (Pearson) correlation if normality was confirmed (Shapiro–Wilk test) and non-parametric (Spearman's rank) correlation otherwise. When comparing seasonal differences in chigger and tick loads on *A. agrarius*, normality and homogeneity of variance were confirmed with Shapiro–Wilk and Levene tests, respectively; data were transformed as necessary. A non-parametric Kruskal–Wallis test was used when neither assumption could be fulfilled and, if required, was followed by a pairwise Mann–Whitney *U*-test after Bonferroni adjustment (α-level: 0.05 divided by n comparisons). Data are given as the mean ± 1 standard error (SE). All procedures were implemented in SPSS Version 16.0 (SPSS, Inc., Chicago, IL, U.S.A.).

Results

Small mammal trapping

Sampling was carried out in 49 plots (5103 trap-nights). A total of 1393 individuals of eight species of small mammal

Table 1. Prevalences and average loads of chiggers among small mammal hosts in central Hua-lien County, eastern Taiwan during 2007/2008.

Host species	Captures, *n*(% of total)	Prevalence of chiggers, %	Chiggers/host, mean ± SE*	Total chiggers, *n* (% of all)
Shrews				
Crocidura attenuata	19 (1.4)	26.32	41.1 ± 30.7	781 (0.7)
Crocidura suaveolens	6 (0.4)	83.33	46.3 ± 19.5	278 (0.3)
Suncus murinus	49 (3.5)	2.04	0.5 ± 0.5	22 (0.02)
Rodents				
Apodemus agrarius	503 (36.1)	97.42	105.3 ± 5.3	52 964 (47.8)
Bandicota indica	40 (2.9)	90.00	288.6 ± 58.1	11 542 (10.4)
Mus caroli	462 (33.2)	0.65	0.01 ± 0.004	3 (0.003)
Mus musculus	231 (16.6)	2.60	0.06 ± 0.04	14 (0.01)
Rattus losea	83 (6.0)	100.00	544.1 ± 49.8	45 156 (40.8)
Total	1393	45.15	79.5 ± 5.3	110 760

*Mean numbers of chiggers/host are calculated across all captures, not just across animals that harboured chiggers.
SE, standard error.

were captured. These included five species of rodent and three species of insectivore (Table 1). The most common species was *A. agrarius* (36.1%), followed by *Mus caroli* Bonhote (33.2%) and *Mus musculus* L. (16.6%) (Rodentia: Muridae). Shrews comprised only 5.3% of total captures.

Prevalences and loads of chiggers among small mammal hosts

A total of 110 760 chiggers were recovered. Their prevalence was very high on *Rattus losea* Swinhoe (100.0%, $n = 83$ hosts), *A. agrarius* (97.4%, $n = 503$) and *B. indica* (90.0%, $n = 40$), but was very low (<2.6%) on *M. caroli* ($n = 462$) and *M. musculus* ($n = 231$). Chigger prevalences on shrews varied from very low (2.0% on *Suncus murinus* L., $n = 49$) to high (83.3% on *Crocidura suaveolens* Pallas, $n = 6$) (both Soricomorpha: Soricidae) (Table 1).

Mean chigger loads were positively associated with chigger prevalence across host species (Spearman correlation $r_s = 0.95$, d.f. $= 7$, $P < 0.01$). *Rattus losea* had the highest load (mean = 544.1 ± 49.8 chiggers/individual), followed by *B. indica* (288.6 ± 58.1 chiggers/individual) and *A. agrarius* (105.3 ± 5.3 chiggers/individual). The other host species had moderate (*C. suaveolens*: 46.3 ± 19.5) to very low (*M. caroli*: 0.01 ± 0.004) chigger loads (Table 1).

The total number of parasites supported by each host species was a function of mean chigger load as well as host population size. The product of these parameters estimates the total chigger population supported by each species; *A. agrarius* hosted the greatest proportion of chiggers (47.8%), although that on *R. losea* was not markedly lower (40.8%). *Bandicota indica* supported only 10.4% of the chiggers retrieved, and the remaining five host species combined hosted <1% of all chiggers (Table 1).

Prevalences and loads of ticks among small mammal hosts

A total of 1431 ticks were collected from 1217 small mammal individuals in 42 plots. Tick prevalence was greatest on *R. losea* (51.9%, $n = 79$), followed by *A. agrarius* (45.0%, $n = 440$) and *B. indica* (28.6%, $n = 35$). The prevalence on the other five host species combined was <6% (Table 2). Mean tick loads increased with tick prevalence ($r_s = 0.97$, d.f. $= 7$, $P < 0.01$), with individual *R. losea* carrying the most ticks (mean = 3.4 ± 1.0 ticks/individual), followed by *A. agrarius* (2.5 ± 0.4 ticks/individual) and *B. indica* (0.8 ± 0.3 ticks/individual). All other hosts supported an average of <0.1 tick/individual (Table 2).

Table 2. Prevalences and average loads of ticks among small mammal hosts in central Hua-lien County, eastern Taiwan during 2007/2008.

Host species	Captures, *n* (% of total)	Prevalence of ticks, %	Ticks/host, mean ± SE*	Total ticks, *n* (% of all)
Shrews				
Crocidura attenuata	19 (1.6)	5.26	0.1 ± 0.1	1 (0.1)
Crocidura suaveolens	6 (0.5)	0	0	0 (0)
Suncus murinus	39 (3.2)	2.56	0.1 ± 0.1	2 (0.1)
Rodents				
Apodemus agrarius	440 (36.2)	45.00	2.5 ± 0.4	1117 (78.1)
Bandicota indica	35 (2.9)	28.57	0.8 ± 0.3	29 (2.0)
Mus caroli	399 (32.8)	1.25	0.02 ± 0.01	9 (0.6)
Mus musculus	200 (16.4)	1.50	0.02 ± 0.01	4 (0.3)
Rattus losea	79 (6.5)	51.90	3.4 ± 1.0	269 (18.8)
Total	1217	21.28	1.2 ± 0.2	1431

*Mean numbers of ticks/host are calculated across all captures, not just across those animals that harboured ticks.
SE, standard error.

Although *A. agrarius* had a lower tick prevalence than *R. losea*, the greater abundance of the former host resulted in a much greater total tick population (78.1% of ticks retrieved). This was followed by that supported by *R. losea* (18.8%). All other host species combined yielded <5% of ticks retrieved (Table 2).

Chigger and tick species composition

In total, 26 274 chiggers were identified, representing roughly a quarter (23.7%) of all chiggers recovered. Among these, 1385 chiggers (5.3% of those identified) could not be keyed (mainly because they were mounted on the slides in positions unsuitable for keying) and were thus excluded. Of the remaining 24 889 chiggers, *Leptotrombidium imphalum* Vercammen-Grandjean & Langston was the most abundant species collected (84.5%), followed by *Leptotrombidium deliense* Walch (14.1%). The remaining chiggers (<2%) included other *Leptotrombidium* species, *Walchia* spp. (Trombidiformes: Trombiculidae) and *Gahrliepia* spp. (Trombidiformes: Trombiculidae) (Table 3). With the exception of *M. musculus*, all host species were infested mainly by *L. imphalum* (>80%) (Table 3).

Rhipicephalus haemaphysaloides comprised >99% of 1431 ticks collected. The remaining ticks were *Ixodes granulatus* Supino and *Haemaphysalis* spp. (both Ixodida: Ixodidae) (Table 4). We recovered larvae (67.7%) and nymphs (32.3%) of *R. haemaphysaloides*, but no adults. The distribution of stage classes of *R. haemaphysaloides* was not random across host species; in general, small host species harboured more larvae (*A. agrarius*: 73.6%; *Crocidura attenuate*: 100.0%; *M. caroli*: 88.9%; *S. murinus*: 100.0%), whereas larger species (*R. losea* and *B. indica*) were parasitized with more nymphs (53.2% and 74.1%, respectively). A possible exception to this was the small *M. musculus*, which harboured a single larva and three nymphs.

Seroprevalence of O. tsutsugamushi *and* Rickettsia *among rodent hosts*

Exposure to *O. tsutsugamushi* was noted in 437 rodents in five species. Across all individuals, seropositivity rates were 70.0%. Species with high chigger loads were more likely to be infected with *O. tsutsugamushi* ($r_s = 0.90$, d.f. $= 4$, $P < 0.05$), although *M. musculus*, which had low mean chigger loads, also yielded over 50% infection with *O. tsutsugamushi*. *Bandicota indica*, *R. losea* and *A. agrarius*, which had the

Table 3. Species composition of chiggers among small mammal hosts in central Hua-lien County, eastern Taiwan during 2007/2008.

	Chigger species composition within host species, %					
Host species	*Leptotrombidium imphalum*	*Leptotrombidium deliense*	Other *Leptotrombidium* spp.	*Walchia* spp.	*Gahrliepia* spp.	Chiggers examined, *n*
Shrews						
Crocidura attenuata	86.4	13.6	0	0	0	88
Crocidura suaveolens	91.7	8.3	0	0	0	72
Suncus murinus	100.0	0	0	0	0	5
Rodents						
Apodemus agrarius	83.2	15.7	1.0	0.01	0.1	12 925
Bandicota indica	82.7	14.3	1.0	2.1	0	2490
Mus musculus	0	0	100.0	0	0	1
Rattus losea	86.6	11.9	0.5	0.8	0.3	9308
Total	84.5	14.1	0.8	0.5	0.2	24 889

Table 4. Total number of ticks of each species and stage recovered from small mammal hosts in central Hua-lien County, eastern Taiwan during 2007/2008.

	Rhipicephalus haemaphysaloides			*Ixodes granulatus*			*Haemaphysalis* spp.			
Host species	L, *n*	N, *n*	A, *n*	L, *n*	N, *n*	A, *n*	L, *n*	N, *n*	A, *n*	Ticks examined, *n*
Shrews										
Crocidura attenuata	1	0	0	0	0	0	0	0	0	1
Suncus murinus	2	0	0	0	0	0	0	0	0	2
Rodents										
Apodemus agrarius	819	294	0	0	2	1	1	0	0	1117
Bandicota indica	7	20	0	1	0	0	1	0	0	29
Mus caroli	8	1	0	0	0	0	0	0	0	9
Mus musculus	1	3	0	0	0	0	0	0	0	4
Rattus losea	124	141	0	0	1	3	0	0	0	269
Total	962	459	0	1	3	4	2	0	0	1431

L, larvae; N, nymphs; A, adults.

highest chigger loads, exhibited very high rates of infection with *O. tsutsugamushi* (>83.6%) (Table 5). Rodents were more likely to be infected with the Kato strain (68.6%) than with the Karp (65.9%) or Gilliam (62.2%) strains (Table 5).

A total of 418 rodents were examined for exposure to *Rickettsia* spp. Seropositivity rates were very high (84–100%) and averaged 91.9% across all species (Table 5).

Seasonal variations in chiggers and human cases of scrub typhus

Apodemus agrarius was selected for seasonal comparisons because this species was frequently infested with chiggers, was abundant in most months, and exhibited high individual variability in chigger load (0–918 chiggers/individual). Trapping efforts were divided into bimonthly periods of August and September 2007, October and November 2007, December 2007 and January 2008, and February and March 2008. January–March 2007 was treated as one period. Mean chigger loads on 503 individual *A. agrarius* varied by season (Kruskal–Wallis test, $H = 78.8$, d.f.= 4, $P < 0.001$), increasing by 77% from August/September (mean $= 124.3 \pm 12.2$) to October/November (mean $= 220.6 \pm 19.2$), before declining to the end of March 2008 (Fig. 1). Higher chigger loads were documented in January–March 2007 (mean $= 96.1 \pm 12.9$) than at 1 year later (mean $= 59.2 \pm 6.6$). Bonferroni-adjusted Mann–Whitney U-tests (10 tests, $\alpha = 0.005$) revealed significantly higher chigger loads during October/November 2007, followed by August/September 2007. The other three periods were similar in chigger loads (Fig. 1).

Human cases of scrub typhus (corrected for latent period) in Hua-lien County demonstrated a bimodal pattern from January 2007 to March 2008, with peaks in June/July 2007 and in October/November 2007 (Fig. 1). For the periods in which chigger populations were quantified, the mean chigger loads on *A. agrarius* were strongly associated with the number of human cases of scrub typhus ($r_s = 1.00$, d.f. $= 4$, $P < 0.01$) (Fig. 1).

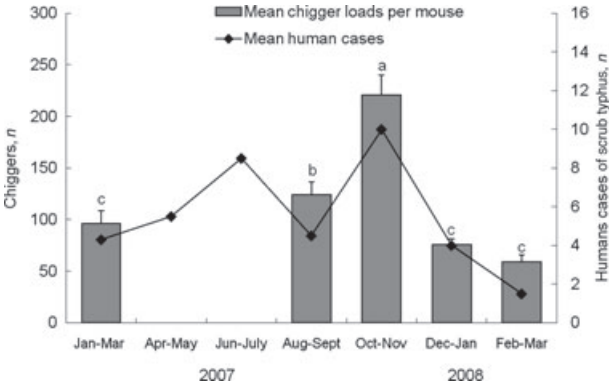

Fig. 1. Seasonal variations in numbers (mean ± 1 standard error) of chiggers recovered from *Apodemus agrarius* and numbers of scrub typhus human cases (by month) in Hua-lien County, eastern Taiwan during 2007/2008. Different letters above the bar indicate significant differences ($P < 0.05$ after Bonferroni adjustment).

Seasonal variations in ticks

As with chiggers, analyses for tick infestations were focused on *A. agrarius* because this species was both abundant and frequently infested with ticks, and exhibited high degrees of variation in tick loads. Trapping periods were divided into the five periods described above except that ticks were collected in March 2007 instead of January–March 2007.

Mean tick loads varied seasonally among 440 *A. agrarius* ($H = 104.2$, d.f. $= 4$, $P < 0.001$), with much higher loads in October/November 2007 (mean $= 10.3 \pm 2.5$), followed by February/March 2008 (mean $= 2.8 \pm 0.4$) and March 2007 (mean $= 1.1 \pm 0.3$) (Table 6). Both larval and nymphal *R. haemaphysaloides* occurred in all five periods, but there was a higher proportion of larvae infesting *A. agrarius* from October to January (85.6–88.1%). The other three periods were dominated by nymphs (55.0–83.9%) (Table 6).

Discussion

The very high seropositivity rates among rodent hosts (*O. tsutsugamushi*: 70.0%; *Rickettsia*: 91.9%) may reflect a lack of specificity in the antigen slides we applied. Findings in studies on the seroprevalences of rodents in other parts of Taiwan using the same materials and methods as in this study (1 : 40 dilution), however, suggest this may not be the case (Fang & Raoult, 2003). Seropositivity rates against

Table 5. Immunofluorescent antibody assay seroprevalences of *Orientia tsutsugamushi* and *Rickettsia conorii* among rodent hosts in central Hua-lien County, eastern Taiwan during 2007/2008.

	Orientia tsutsugamushi					*Rickettsia conorii*	
		Prevalence, %					
Host species	Tested, *n*	Kato	Karp	Gilliam	Overall	Tested, *n*	Prevalence, %
Apodemus agrarius	231	81.8	81.8	76.6	83.6	226	94.7
Bandicota indica	18	100.0	94.4	94.4	100.0	17	100.0
Mus caroli	93	23.7	17.2	14.0	25.8	91	84.6
Mus musculus	50	56.0	50.0	50.0	56.0	43	83.7
Rattus losea	45	95.6	91.1	91.1	95.6	41	97.6
Total	437	68.6	65.9	62.2	70.0	418	91.9

Table 6. Seasonal variations in average tick loads and percentages of larvae and nymphs recovered from *Apodemus agrarius* in central Hua-lien County, eastern Taiwan during 2007/2008.

Time period	Hosts examined, *n*	Tick loads, mean ± SE*	Percentage within period	
			Larvae	Nymphs
March 2007	55	1.1 ± 0.3[bc]	16.1	83.9
August/ September 2007	82	0.5 ± 0.1[c]	41.5	58.5
October/ November 2007	63	10.3 ± 2.5[a]	88.1	11.9
December 2007/January 2008	162	0.9 ± 0.2[c]	85.6	14.4
February/March 2008	78	2.8 ± 0.4[b]	45.0	55.0

*Means followed by different letters are significantly different ($P < 0.05$). SE, standard error.

O. tsutsugamushi ranged from 0% to 81.6% across different localities in Taiwan; similarly, seropositivity rates against *R. conorii* or *R. rickettsii* can be as low as 30% in some parts of Taiwan, where proof of exposure for some species (excluding *A. agrarius*) cannot even be detected (0%) (H. C. Wang, unpublished data, 2010). Wu (2006) documented seropositivity rates against *R. conorii* of 20.0–55.6% in rodents and shrews in different localities of the Ma-tsu Islands. In this study, a lower serum dilution relative to that for human assay (1 : 64; Focus Technologies, Inc.) was selected because the ultimate purpose of studying seroconversion in wild rodents is to identify potential human exposure to disease infection. Nevertheless, because seroprevalence will depend on the level of dilution selected (i.e. a 1 : 80 dilution will generate lower positive rates than a 1 : 40 dilution), the current study is reserved in interpreting the very high seropositivity rates as indicative of the exact degree of exposure of rodent hosts to *O. tsutsugamushi* and *Rickettsia* spp. Instead, we consider that the method used in the current study may reflect the 'relative' seropositivity rates across different localities and rodent species and that the current data reveal a frequent enzootic transmission of *O. tsutsugamushi* and tick-borne SFG rickettsiae in central Hua-lien in eastern Taiwan.

The chigger assemblages were composed primarily of *L. imphalum*. This species was also identified as the predominant chigger in northern Thailand (41%), where it infested 15 of 16 small mammal species (Coleman *et al*., 2003). By contrast, *L. deliense* was found to be the most common and widespread chigger species in Southeast Asia, the southwest Pacific islands and parts of China (Kawamura *et al*., 1995). *Leptotrombidium deliense* was also dominant in several parts of Taiwan, including the Penghu Islands and Kin-men Islands (summer period only)(Olson *et al*., 1982; Wang, 2004). It is not clear why a different species of chigger dominates in central Hua-lien. Because small mammals were not sampled from April to July, it is not possible to rule out the possibility that *L. deliense* may have dominated during this period. In Kin-men, *L. deliense* was replaced by *Leptotrombidium scutellare* Nagayo, Mitamura, Tamiya & Tenjin between December and April, when temperatures were <20 °C (Wang *et al*., 2004). However, because temperatures were well >20 °C during most of the study period (except January 2007 and January/February 2008), we do not believe that the absence of *L. deliense* represents a sampling artefact.

It is also possible that changes in the host community are associated with changes in the parasite fauna. Whereas *A. agrarius* was the principal host in our study area, *Rattus* spp. are dominant hosts elsewhere in Taiwan (Olson *et al*., 1982; Wang, 2004). Because the distribution of *A. agrarius* in Taiwan is very restricted and these animals occur mostly in small numbers (Chu, 2000), this study may demonstrate a unique host–chigger dynamic; if so, this dynamic should be further studied to provide better understanding of the high prevalence of scrub typhus in this part of Taiwan.

Larval and nymphal, but not adult, *R. haemaphysaloides* were recovered from small mammals. It is likely that rodents are not the main hosts for adult *R. haemaphysaloides*. In Taiwan, adult *R. haemaphysaloides* have been collected from dogs, cattle and goats, but rarely from rodents (Tsai, 2002; H. C. Wang, unpublished data, 2009). Adults of this species have also been recovered from medium and large mammals elsewhere, including cattle, sheep, goats, camels, horses, wild carnivores and ungulates (Robbins *et al*., 1997; Walker *et al*., 2000; Grassman *et al*., 2004; Latha *et al*., 2004; Durden *et al*., 2008); however, most of these studies did not target small mammals and thus the lack of data on infestations does not indicate the absence of such infestations. Mammals are the main hosts for *Rhipicephalus* spp., which rarely feed on birds and never parasitize reptiles (Oliver, 1989). Because dogs and livestock are rare and wild ungulates and carnivores do not exist in our study site, hares [*Lepus sinensis* Gray (Lagomorpha: Leporidae)] are one of the few likely hosts for adult *R. haemaphysaloides*. Ectoparasites of hares have rarely been studied in Taiwan, but hares have been reported as hosts for mature *R. haemaphysaloides* elsewhere (Walker *et al*., 2000). Elucidating the role of hares or other large vertebrates in maintaining adult *R. haemaphysaloides* may facilitate the design of strategies to control tick-borne diseases through the proper management of host populations.

By documenting the main chigger and tick species in eastern Taiwan, as well as the host species for these parasites, the current study represents the first step towards identifying key players in the enzootic transmission of *O. tsutsugamushi* and tick-borne SFG rickettsiae in eastern Taiwan. It is tempting to extrapolate from these data in order to make inferences about the role of more common species here (e.g. the parasites *L. imphalum* and *R. haemaphysaloides*, and the hosts *A. agrarius* and *R. losea*). Before this is possible, however, the presence of pathogens in these chiggers and ticks should be confirmed (Tanskul *et al*., 1998; Tsui *et al*., 2007). Equally important is the evaluation of the proclivity of both vectors to humans. Because chiggers are so small, victims of scrub typhus are often unaware of any chigger infestation, which renders the recognition of vectors difficult, but cumulative studies of chiggers in localities in which patients may be exposed to

them should help to confirm the vector species. Similarly, the frequency with which humans are infected by ticks in Taiwan is poorly understood. Efforts to report tick infestation and submit tick specimens should be encouraged so that the proclivity of tick species to humans in Taiwan can be better characterized.

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