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Spatial analysis of scrub typhus infection and its association with environmental and socioeconomic factors in Taiwan

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ABSTRACT

We analyzed the spatial distribution of human cases of scrub typhus on the main island of Taiwan from 2003 to 2008 and implemented an island-wide survey of scrub typhus vectors (trombiculid chiggers) in 2007 and 2008. The standardized incidence rate 'SIR' incorporating inter-district variations in population, gender and age was correlated with environmental and socioeconomic variables. Higher incidence and SIR rates were clustered in the less developed, mountainous regions of central and eastern Taiwan. Higher SIRs were also associated with a higher proportion of dry-field farmers in the population, a higher normalized difference vegetation index (NDVI) and lower mean annual temperature, but was not associated with rainfall. Small mammal hosts in high-SIR districts harbored more chiggers and had higher rates of seropositivity against *Orientia tsutsugamushi* Hyashi, the etiologic agent of scrub typhus, compared to low-SIR districts. The concurrence of a higher proportion of dry-field farmers and higher NDVI has likely led to the clustering of scrub typhus in the mountainous regions of Taiwan. Further individual-level study of the risk factors associated with scrub typhus, and a better understanding of the effect of environmental factors on chigger abundance, should help to prevent scrub typhus in Taiwan.

1. Introduction

Scrub typhus, caused by *Orientia tsutsugamushi* Hyashi (OT), is an acute human infectious disease that is prevalent in the western Pacific, South Asia, and northeastern Australia. Transmitted by larval trombiculid mites (i.e., chiggers), this disease infects approximately one million people annually, and one billion more are estimated to be at risk (Kawamura et al., 1995; Rosenberg, 1997). The life cycle of trombiculid mites includes seven stages (egg, deutovum, larva (chigger), protonymph, deutonymph, tritonymph, and adult), of which only chiggers are parasitic. The free-living nymphs and adults live on the ground and feed mainly on arthropod eggs and larvae (Kawamura et al., 1995). *Leptotrombidium* sp. chiggers are the primary vectors and murine rodents are the predominant hosts of chiggers in regions endemic for scrub typhus (Kawamura et al., 1995; Traub and Wisseman, 1974).

The occurrence of scrub typhus is frequently related to temperature, and sometimes to rainfall (Kawamura et al., 1995; Olson, 1979; Olson and Scheer, 1978; Traub and Wisseman, 1974). The relationship between human incidence of scrub typhus and climate should largely reflect the responses of chiggers to the environment (Kawamura et al., 1995). In the Pescadores Islands (Taiwan), the abundance of *L. deliense* was positively correlated with temperature (Van Peenen et al., 1976), and in Malaysia, *L. deliense* and *L. akamushi* were sensitive to changes in temperature and humidity (Gentry et al., 1963). Chigger abundance may be primarily influenced by temperature in temperate areas but by precipitation in tropical regions (Gentry et al., 1977). The habitats and altitudinal distribution of chiggers varied by species, but they were more likely to be located in areas with moist soil and abundant rodents (Traub and Wisseman, 1974). Exposure to scrub typhus may also be related to socioeconomic factors. Increased urbanization and higher school enrollment rates may contribute to decreased incidence among children in the Pescadores Islands (Olson and Bourgeois, 1979), and the incidence in Japan and eastern Taiwan was greater among farmers than among people with other occupations (Lee et al., 2006; Ogawa et al., 2002).

Taiwan is a moderately sized island (36,000 km^2) off the coast of China. The eastern two-thirds of Taiwan are characterized by rugged mountains covered with tropical and subtropical vegetation. Western Taiwan is characterized by flat to gently rolling plains. Scrub typhus was first reported in Taiwan in 1908 (Hatori, 1919) and is currently the most common rickettsial disease in the country (Tsai et al., 2008). On the main island of Taiwan, most human cases

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occur in eastern Taiwan (Lee et al., 2006), but the reason for this geographical distribution is poorly understood. There is a similar lack of research in other countries where scrub typhus is prevalent (e.g., China, Japan, Korea, and Thailand). A better understanding of the spatial distribution of the incidence along with the possible underlying causes of the distribution can allow for more targeted disease control efforts and assist in the prediction of disease dynamics. For instance, global warming could affect the spatial range and incidence of scrub typhus if temperature is the main regulator. The recent emergence of this disease (Cao et al., 2006; Kweon et al., 2009) has underscored the importance of understanding these factors. Moreover, the lack of disease may reflect the absence of the pathogen rather than the lack of a suitable environment for the vectors. Elucidating this is important to forecast disease emergence in the face of environmental change.

We studied the spatial distribution of scrub typhus incidence in Taiwan from 2003 through 2008. After identifying regions with high incidence rates, we analyzed the association between incidence and commonly available environmental and socioeconomic factors. Regions with low or high incidence rates were also surveyed for chiggers. Specifically, we investigated whether chiggers were absent or rare in the low-incidence regions or whether the incidence rate was more significantly determined by the infection rate of OT. To the best of our knowledge, this is the first large-scale study featuring a systematic survey of chigger distribution and the use of sophisticated statistical methods to explore possible causal factors in the spatial variation in scrub typhus incidence in Taiwan and other countries.

2. Materials and methods

2.1. Study area

This study focused on the main island of Taiwan. We excluded small associated islands (Kin-men, Ma-tou, Pescadores, Little Liuchiu, Green and Orchid islands) because they frequently differ with regard to potentially important ecological characteristics (e.g., animal communities; Courchamp et al., 2003). The basic geographical units used in this analysis were administrative districts (within urban cities) and townships (within rural counties) because these are the smallest administrative areas within which scrub typhus infection can be recorded (see Section 2.2). In this study, the term “district” is used to refer to both the urban districts and the rural townships.

2.2. Disease incidence

Scrub typhus is a notifiable disease in Taiwan. Blood samples from patients with suspected scrub typhus are collected and sent to the Taiwan Centers for Disease Control (CDC) for laboratory diagnosis. Samples are considered positive for scrub typhus based on a positive real-time polymerase chain reaction (PCR) test or the detection of OT-specific antibodies based on the indirect immunofluorescent assay (IFA) (four-fold increase in OT-specific immunoglobulin M (IgM) or IgG antibody in paired sera). Because infection may occur away from a patient’s residence, starting in 2003, the presumptive location of infection was added to the list of required information in these reports. These data, as well as the gender and age of patients, are available from the Taiwan CDC. To more accurately assess the relationship between infection and environmental factors, we allocated incidence rates of scrub typhus (2003–2008) to the district in which the infection occurred rather than the district in which the patient resided.

Human disease risk often varies with age and gender, and inter-district variation in incidence can be due to differences in age structure or sex ratio between districts rather than to underlying environmental variations (Waller and Gotway, 2004). We applied chi-square tests to determine whether the incidence rate (# of cases/population size/year) differed between age groups (0–9, 10–19. . .80–89, >90 years old) and between males and females across the entire study population. Both age and gender had significant influence on incidence rates (see Section 3.1), so we corrected for possible inter-district variation in age structure and sex ratio by standardizing our data (Waller and Gotway, 2004), yielding standardized incidence rates (SIRs) for each district. The SIR was the primary variable for subsequent spatial and regression analyses. We obtained the population size for each age and gender group (mean value from 2003 to 2008) in all districts from the Department of Statistics of the Taiwan Ministry of the Interior.

2.3. Environmental and socioeconomic variables

We selected variables for analysis based on the availability of data and our knowledge of the study system. Predictive environmental variables included total annual rainfall (Rainfall, mm), mean annual temperature (Temp, °C; calculated as the mean of 12 monthly mean temperatures), and the normalized difference vegetation index (NDVI). We compiled a long-term (1959–1985) temperature and rainfall dataset from the Taiwan Central Weather Bureau. The two climatic variables were stored in a Geographic Information System dataset with a spatial resolution of 1 km × 1 km (Lee et al., 1997). The NDVI was calculated using mosaic data collected by SPOT (Satellite Pour l’ Observation de la Terre, France) satellites. The NDVI provides a measure of photosynthetically active vegetation and was selected as an indicator of land use. The NDVI is positively correlated with the degree of vegetation growth, with low values (close to 0) reflecting barren habitat and high values (close to 1) reflecting densely forested habitat (Box et al., 1989; Kerr and Ostrovsky, 2003; Pettorelli et al., 2005); as such, we treated higher NDVI values as representing areas with less human development. NDVI values were calculated once a year between 2002 and 2007. Nevertheless, the frequently cloudy climate in Taiwan made accurate NDVI data for the whole island unavailable for most years. Accordingly, the NDVI data for 2002 were selected for analysis because they were of better quality. NDVI data were also stored with a spatial resolution of 1 km × 1 km. We overlaid political district boundaries in these 1 × 1-km grids to calculate temperature, rainfall, and NDVI for each district using ArcGIS 9.3 (ESRI, Redlands, CA), and we used the average of the overlaid grids to determine these parameters for each district. Although these environmental variables were collected outside of the period when the incidence of scrub typhus was compiled, a correlation study of these variables should still be valid (e.g., a district with relatively low temperature before the period of incidence data collection should still have relatively low temperatures). Additionally, significantly large-scale (district-wide) changes in NDVI between 2002 and 2008 were unlikely to occur due to strict land use regulations in most parts of Taiwan.

To assess the role of socioeconomic factors, we recorded the proportion of farmers in the population (“Farmers” variable) within districts. Because many farmers in Taiwan work in flooded rice paddies, where flood-susceptible chiggers have low survival rates (Kawamura et al., 1995), we also defined the “Dry_farmers” variable as the product of the “Farmers” variable and the proportion of cultivated lands within districts that were dry, both of which were represented by the mean value from 1996 to 2004 (acquired from the Taiwan Council of Agriculture).

2.4. Spatial clusters of disease incidence

We assessed spatial clustering of SIR using Moran’s I (Moran, 1950). If spatial autocorrelation was found, we assessed the location of SIR clusters using local indicators of spatial association (LISAs) (Anselin, 1995). LISAs can be treated as a local version of Moran's *I*, and they can be used to detect clusters of observations with similar or dissimilar values (Anselin, 1995). Based on a permutation of observed SIR values, each district was categorized as having a low (below designated threshold of significance), high (above threshold of significance), or non-significant SIR (Anselin, 1995). For each district, all adjacent districts were then permuted in a similar manner to assess whether the mean of these SIR values was low, high, or not significant. Based on these values, a map of LISA clusters allowed the assignment of each district to one of five categories as follows: high-high, which indicates a district with high SIR surrounded by districts with high mean SIR (also called a hot spot); low-low, which indicates a district with low SIR surrounded by low-SIR districts (a cold spot); low-high, which indicates a district with low SIR surrounded by high-SIR neighbors; high-low, which indicates a district with high SIR surrounded by low-SIR neighbors; and not significant, which indicates a district with no significant autocorrelation (Anselin, 2003). Inference for significance of both Moran's *I* and LISAs was based on 1999 permutations using the GeoDa 0.9.5 software (Anselin et al., 2006). The threshold of significance was set at $P = 0.05$, and maps are displayed with ArcGIS 9.3.

2.5. Relationship between incidence rate and explanatory variables

We applied Spearman rank correlations to assess the relationships between SIR and five explanatory variables (Temp, Rainfall, NDVI, Farmers, and Dry_farmers). We dropped highly correlated explanatory variables ($r_s > 0.7$; Hu et al., 2007; Winters et al., 2009) and retained the variable most closely related to SIR in an ordinary least square (OLS) regression. We corrected for high spatial autocorrelation in the OLS regression models (based on Moran's *I*, data not shown) using a spatial error model that incorporated neighboring residuals to remove spatial autocorrelation (Anselin and Bera, 1998; Legendre, 1993). When fitting the OLS regression and subsequent spatial models, SIR was transformed to normalize the data and stabilize the variance: new SIR = $\log_{10}(\text{SIR} + (100{,}000/\text{population}))$ (Waller and Gotway, 2004). Explanatory variables were log transformed as needed to fulfill the assumptions of normality and homogeneity of variance. Normality of error and homogeneity of variance were assessed with the Shapiro–Wilk and Breusch–Pagan tests, respectively.

The importance of explanatory variables was determined by model selection and based on Akaike's information criterion (AIC). The model with the lowest AIC score ($\text{AIC}_{\min}$) and those with AIC differences ($\Delta\text{AIC} = \text{AIC}_i - \text{AIC}_{\min}$ for model *i*) less than 2 ($\Delta\text{AIC} \le 2$) were considered to be the best-supported models (Burnham and Anderson, 2002). The relative weight of evidence among the candidate models can be represented by the Akaike weights ($w_i = \exp(-0.5\Delta\text{AIC})/\text{sum of} \exp(-0.5\Delta\text{AIC})$ for all i models), which sum to 1.0 across all models. Higher values of w_i indicate greater support for a given model (Burnham and Anderson, 2002). Based on the Akaike weights, we also estimated (1) the relative importance of explanatory variables ($w_+(j)$ for variable *j*), which is determined as the sum of w_i over the candidate models containing variable *j*; (2) the parameter coefficient (θ_j), which was estimated by model averaging as the sum of $w_i{}^*\theta_j$ across the subset models containing variable j divided by the sum of w_i, and was applied to assess the direction of correlation with the SIR (i.e., negative or positive) (Burnham and Anderson, 2002). All statistical procedures were conducted using SPSS 16.0 (SPSS Inc. Chicago, Illinois) and GeoDa 0.9.5 (Anselin et al., 2006).

2.6. Island-wide small mammal trapping and collection of chiggers

We surveyed chiggers in low-lying lands (<500 m in elevation) and agricultural fields that were abandoned in the summer (June to August) of 2007 and 2008; the months of June through August were the period of peak incidence of scrub typhus in Taiwan between 1998 and 2006 (Kuo et al., unpublished results). Districts with low (<mean SIR calculated from 2003 to 2006) and high (>mean) SIR were sampled alternatively to control for any seasonal influence. Because of the inefficiency of sampling for chiggers in Taiwan (Wang et al., 2005), we instead compared the abundance of chiggers recovered from rodents. In each study site, we deployed five Sherman traps (26.5 cm × 10 cm × 8.5 cm) and 10 meshed live traps (27 cm × 16 cm × 13 cm). Meshed traps were used to target less abundant, but larger, lesser rice-field rats (*Rattus losea* (Swinhoe)) and greater bandicoot rats (*Bandicota indica* (Bechstein)). Traps were opened and baited with sweet potato covered with peanut butter in the evening and they were checked for captures early in the morning. All sites were surveyed for one night.

Trapped small mammals (rodents and shrews) were transferred to a clean nylon mesh bag; bags were carefully examined to ensure that no ectoparasites remained from earlier captures. Rodents were anesthetized with Zoletil 50 (Fa. Virbac. Carros, France) and examined for ectoparasites by combing their fur thoroughly. Areas of skin with attached chiggers were detached carefully using tweezers and preserved in vials; the chiggers detached themselves from the skin and were transferred to 70% ethanol after two days. Chiggers recovered from animals were counted individually. Approximately 0.1 ml of blood, collected from the submandibular area or from the saphenous vein, was centrifuged and sera were stored at −70 °C for later evaluation. Rodents were then released at the trapping sites. Shrews were initially screened for ectoparasites. Shrews infested with chiggers were euthanized with an overdose of Zoletil 50 and blood was collected via heart puncture. All procedures were approved by the Animal Use and Care Administrative Advisory Committee, the University of California, Davis, and met guidelines recommended by the American Society of Mammalogists (Gannon et al., 2007).

2.7. Immunofluorescent antibody assay (IFA)

We assayed rodents for OT exposure using the IFA method. Each serum sample was diluted 1:40 in phosphate-buffered saline (PBS) and applied to antigen-coated slides (Gu-Yuan Biotech. Ltd., Taiwan). The slides were then incubated 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, USA) 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 cover slips. We used sera from wild rodents with PCR-confirmed OT infection in their livers, spleens, and kidneys as positive controls and used sera from rodents without infection as negative controls. Assays were considered positive when bright green fluorescence matched the fluorescence of positive controls based on fluorescence microscopy (Leica, Wetzlar, Germany). The OT antigen slides allowed for simultaneous screening of three strains (Kato, Karp, and Gilliam). Serum samples were scored as negative for OT when

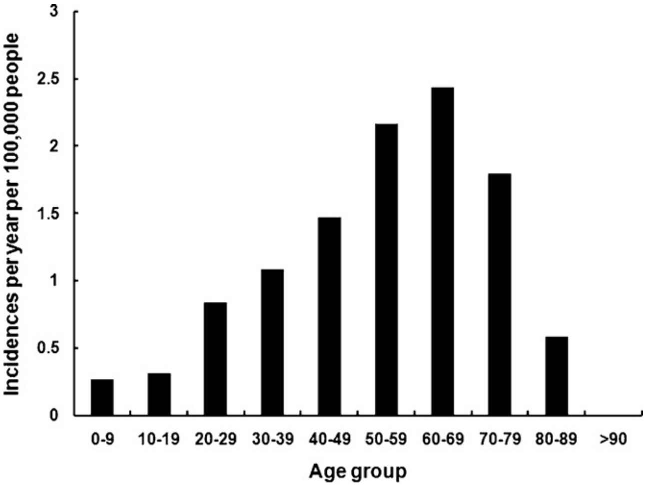

Fig. 1. Age-specific incidence of scrub typhus (per year per 100,000 residents) from 2003 to 2008 on the main island of Taiwan.

the results for all three strains were negative; if the test for any strain yielded a positive result, the sample was recorded as positive.

3. Results

3.1. Disease incidence

From 2003 to 2008, a total of 1558 confirmed scrub typhus infections were reported on the main island of Taiwan. The mean incidence rate was 1.15 cases per year per 100,000 residents, and it was significantly higher for males than for females (1.43 vs. 0.85; Chi-square test with Yates' correction, $\chi^2 = 100.8$, $P < 0.001$). Incidence also varied by age group ($\chi^2 = 573.0$, $P < 0.001$), increasing monotonically in the 60–69 age range (2.43) and subsequently declining (Fig. 1).

3.2. Spatial clusters of disease incidence

In 350 administrative districts, the cumulative incidences during the study period varied from 0 to 54 cases. Incidences were not distributed evenly across Taiwan; more human cases occurred in central and eastern Taiwan (Fig. 2A), especially in Hua-lien, Tai-tung, and Nan-tou counties. A high number of cases were also reported in parts of southwestern Taiwan (Kaohsiung city), whereas very few cases occurred in northern and western Taiwan (Fig. 2A).

The SIR varied from 0 to 64.2 cases per year per 100,000 residents. As with the raw (unstandardized) data, the SIR was higher in central and eastern Taiwan (Fig. 2B).

SIRs were spatially clustered (Moran's $I = 0.70$, $P < 0.0001$). A map of LISAs revealed high SIRs in central and eastern Taiwan (high-high, Fig. 2C), whereas low SIRs were more commonly found in northern and western Taiwan, especially in regions along the coast (low-low, Fig. 2C). There were also two districts in central Taiwan and one district in southwestern Taiwan that had a low SIR but were surrounded by districts with high SIRs (low-high, Fig. 2C).

3.3. Relationship between environmental and socioeconomic variables and SIR

The values of explanatory variables varied among the analyzed districts; mean temperature (Temp): 13.9–24.8 °C, total rainfall: 1204–4861 mm, NDVI: <0.01–0.53, Farmers: 0–86.0%, and Dry_farmers: 0–85.4%.

SIR was significantly correlated with all the explanatory variables (Table 1). All the variables were positively correlated with SIR except Temp, which was negatively correlated. A strong correlation was also observed between the explanatory variables. The variable Farmers was dropped from subsequent analyses due to a high correlation with Dry_farmers ($r_s > 0.7$) (Table 1). The spatial error model including the Dry_farmers, NDVI, and Temp variables provided the best fit to our data based on AIC and w_i (0.791) (Table 2), and it explained most of the variation in SIR ($R^2 = 0.839$). All other models had AIC differences (ΔAIC) well over 2 (Table 2). These three variables also had the highest relative impor-

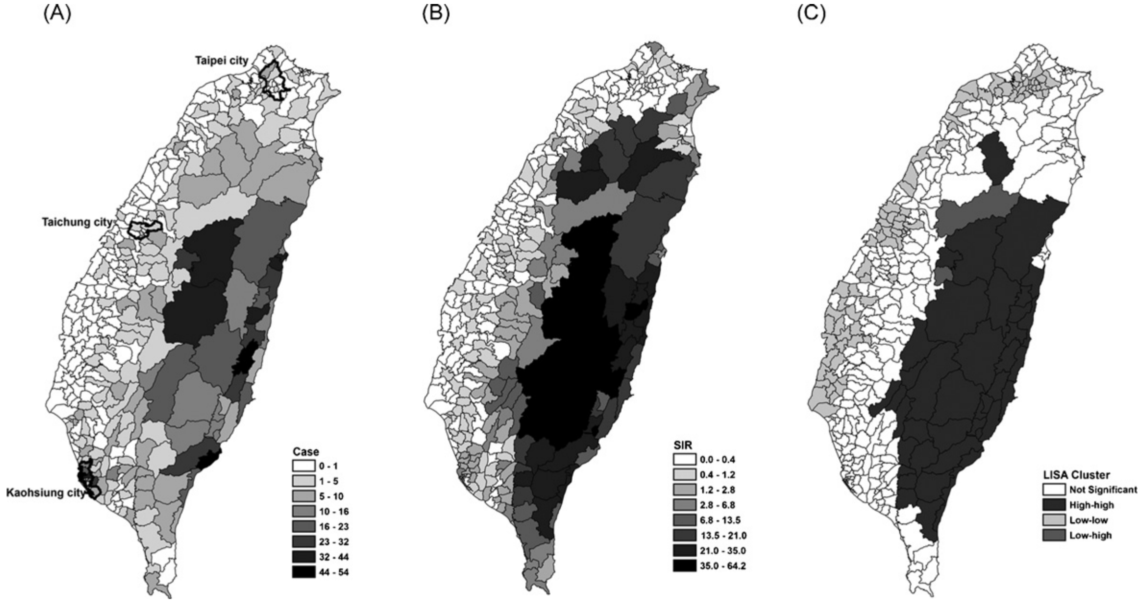

Fig. 2. Spatial variation in the occurrence of scrub typhus from 2003 to 2008 in 350 administrative districts in Taiwan: (A) cumulative incidence, (B) standardized incidence rate (SIR, cases per year per 100,000 residents), (C) local indicators of spatial association (LISAs), see Section 2.4 for detailed descriptions of map legends. Note that no district was characterized as "high-low." The significance threshold of clusters was set at $P < 0.05$.

Table 1
Spearman rank correlation coefficient (r_s) between scrub typhus SIR (standardized incidence rate) from 2003 to 2008 on the main island of Taiwan and environmental and socioeconomic variables (Temp: mean annual temperature; NDVI: normalized difference vegetation index; Rainfall: total annual rainfall; Farmers: proportion of farmers in the population; Dry_farmers: product of Farmers and proportion of dry cultivated lands).

	SIR	Temp	NDVI	Rainfall	Farmers
Temp	−0.210*				
NDVI	0.510*	−0.631*			
Rainfall	0.438*	−0.618*	0.694*		
Farmers	0.210*	−0.204*	0.422*	0.029	
Dry_farmers	0.444*	−0.277*	0.675*	0.325*	0.782*

* $P<0.001$.

Table 2
Akaike information criterion (AIC) score, AIC difference (ΔAIC) and Akaike weight (w_i) for competing models after fitting the spatial error model with SIR as the dependent variable (refer to Table 1 for the meaning of acronyms).

Model[a]	AIC	ΔAIC	w_i
Dry_farmers + NDVI + Temp	−66.69	0	0.791
Dry_farmers + NDVI + Temp + Rainfall	−62.82	3.87	0.114
Dry_farmers + NDVI	−60.90	5.79	0.044
Dry_farmers + Temp	−59.61	7.08	0.023
Dry_farmers + NDVI + Rainfall	−58.92	7.77	0.016
Dry_farmers + Temp + Rainfall	−57.63	9.06	0.009
Dry_farmers	−54.54	12.15	0.002
Dry_farmers + Rainfall	−53.14	13.55	0.001
NDVI + Temp	−7.44	59.25	<0.0001
NDVI + Temp + Rainfall	−5.46	61.23	<0.0001
Temp	6.85	73.54	<0.0001
Temp + Rainfall	7.91	74.6	<0.0001
NDVI + Rainfall	14.19	80.88	<0.0001
NDVI	16.22	82.91	<0.0001
Rainfall	31.22	97.91	<0.0001

[a] NDVI and Temp were log-transformed.

tance (Dry_farmers, $w_+ = 1.00$; NDVI, $w_+ = 0.97$; Temp, $w_+ = 0.94$) (Table 3). The Dry_farmers and NDVI variables were positively correlated with SIR, whereas Temp was negatively associated with SIR (Table 3).

Table 3
Relative importance of explanatory variables (w_+) and variable parameter coefficient (θ) from model averaging after fitting the spatial error model with SIR as the dependent variable (refer to Table 1 for the definitions of acronyms).

Variable	w_+	θ
Dry_farmers	1.00	0.96
Log(NDVI)	0.97	0.30
Log(Temp)	0.94	−1.15
Rainfall	0.14	-8.30×10^{-6}

3.4. *Abundance of chiggers and seroprevalence of OT in low- vs. high-SIR districts*

A total of 103 sites in 92 districts were surveyed, including 38 sites in 36 low-SIR districts and 65 sites in 56 high-SIR districts (Fig. 3). We trapped 192 small mammals, including two species of shrews and five species of rodents (Table 4). Mean abundances of chiggers in *Crocidura attenuata* Milne-Edwards, *Suncus murinus* L., *Mus caroli* Bonhote, and *M. musculus* L. were similar in both types of districts, but *Apodemus agrarius* Pallas, *B. indica*, and *R. losea* harbored many more chiggers in high-SIR than in low-SIR districts (Table 4). A total of 44 rodents were assayed for OT exposure using the IFA method. Seropositivity rates in *A. agrarius*, *B. indica*, and *R. losea* were much higher in high-SIR than in low-SIR districts (Table 4).

4. Discussion

Between 2003 and 2008, there were more scrub typhus infections in central and eastern Taiwan, which is less developed and more mountainous than northern and western Taiwan. These clusters remained after accounting for population size (e.g., using incidence rates). Similar geographic variation in the incidence of scrub typhus in Taiwan has been reported since 1908 (Hatori, 1919; Lee et al., 2006; Wang, 1988), and outbreaks of this disease had been documented only in the eastern part of the main island (Gale et al., 1974). Such spatial variation in incidence may reflect regional difference in diagnosis and reporting of diseases, but the higher occurrence of scrub typhus in remote central and eastern Taiwan, where hospitals are less accessible, should still reflect a real excess of disease in these regions.

Although most cases of scrub typhus occurred in rural Taiwan, many cases were also reported in the densely populated city of Kaohsiung in southwestern Taiwan (Fig. 2A). Although such a high incidence could reflect the high local population density, such high incidence rates were not observed in other large cities in Taiwan

Table 4
Comparison of chigger abundance and seroprevalence of *Orientia tsutsugamushi* (based on immunofluorescent antibody assay, IFA) in small mammal hosts in districts with high vs. low standardized incidence rates (SIRs) of scrub typhus.

Host species	High SIR			Low SIR		
	Number	Chigger load[a]	Seropositive rate[b]	Number	Chigger load	Seropositive rate
Shrew						
Crocidura attenuata	1	0	–	3	5.3	–
Suncus murinus	6	0	–	2	0	–
Rodent						
Apodemus agrarius	24	57.4	73.7% (19)	4	0	0% (1)
Bandicota indica	14	117.4	100% (6)	8	8.7	20% (5)
Mus caroli	53	0	–	8	0	–
Mus musculus	33	0	–	0	0	–
Rattus losea	18	425.3	87.5% (8)	18	6.6	40% (5)
Total	149	71.6	81.8% (33)	43	4.8	27.3% (11)

[a] Total chiggers/host.
[b] Seropositive rate (number used for IFA assay); –: species not assayed.

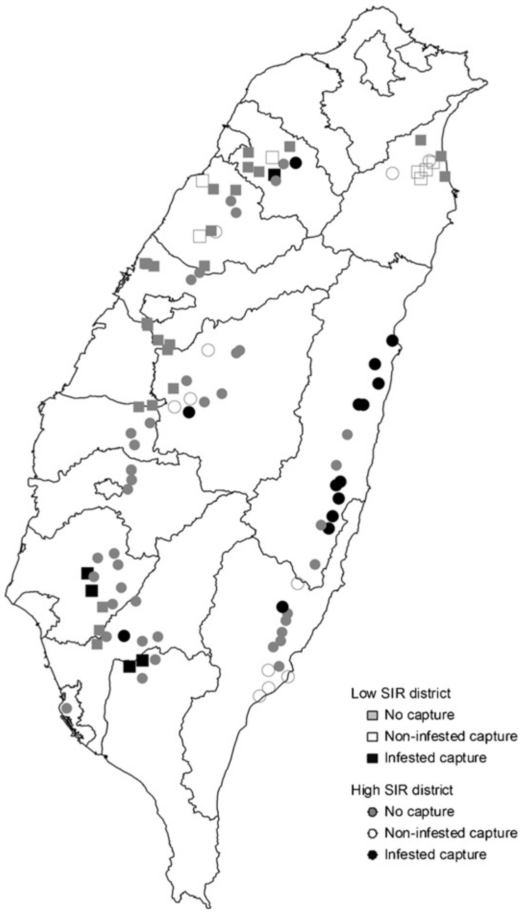

Fig. 3. Locations used for island-wide chigger survey and occurrence of chiggers in small mammal hosts (infested or not, or no capture of hosts) captured in the summers of 2007 and 2008 in districts with low vs. high SIR (administrative boundaries are based on counties).

(e.g., Taipei city, Taichung city, Fig. 2A). Kaohsiung city is notable for the presence of the Shou Mountain at its northwestern border. This mountain is under strict military control and therefore is less developed, likely providing a suitable habitat for chiggers and rodent hosts (Traub and Wisseman, 1974). Local residents frequently use permissible trails on this mountain, and they may get infected with scrub typhus. We tried to trap rodents on this mountain during the island-wide survey but failed to collect any specimen. Determining whether Shou Mountain was a focus of infection therefore requires additional investigation, including an assessment of the abundance and OT infection prevalence of chiggers.

Our analysis demonstrated that broad-scale (inter-district) variation in SIR can largely be explained by NDVI, mean annual temperature, and especially by the proportion of farmers working in dry fields. In both Taiwan and Japan, farmers have a disproportionately higher frequency of infection (Lee et al., 2006; Ogawa et al., 2002), likely due to their frequent exposure to disturbed habitats where chiggers and rodents abound (Traub and Wisseman, 1974). Moreover, SIR was more strongly associated with dry-field farmers than with farmers in general (Table 1). This may reflect the fact that a high proportion of farmers in Taiwan, especially in western Taiwan, work in flooded rice paddies, where flood-susceptible chiggers survive poorly (Kawamura et al., 1995). Rice farmers may thus be less susceptible to scrub typhus than farmers working in dry fields. In support of this explanation, scrub typhus in Korea was observed to be more common in women than in men; this was attributed in part to the fact that most men worked in rice paddies, whereas women worked in dry fields (Kweon et al., 2009). A similar gender difference in working habitats was not observed in Taiwan, and men generally spent more time outdoors than women, likely contributing to a higher incidence rate in men than in women in Taiwan.

It has been recognized that the incidence of scrub typhus is higher in secondary vegetation, in the vicinity of streams or intact forests, and in vegetation recently recovered from human disturbance, where chiggers and small mammal hosts are more abundant (Traub and Wisseman, 1974). Scrub typhus has rarely been reported in urban regions (Matsui et al., 2002), where vegetation cover is greatly diminished. This pattern is supported by the positive relationship between incidence of scrub typhus and NDVI, an index that varies in accordance with the degree of vegetation growth.

The incidence of scrub typhus is usually seasonal, especially in temperate regions. Incidence peaks in summer, although some less virulent strains peak mainly in winter (Kawamura et al., 1995). The negative association between mean annual temperature and SIR in this study was contrary to our expectation. This may reflect a direct influence of mean temperature on SIR, although it is more likely that SIR is influenced by other factor(s) that co-vary with mean annual temperature, such as NDVI, which was also correlated with SIR (Table 1). The mountainous topography of much of Taiwan complicates this analysis, as most environmental factors are highly correlated with elevation; in particular, regions of higher elevation have not only higher NDVIs (due to less human disturbance) but also lower temperatures (Koh et al., 2006; Lee et al., 2004). Dissecting the relative importance of NDVI vs. mean temperature is therefore difficult. Not surprisingly, a model with the Dry_farmers and NDVI variables had very similar AIC scores as a model with the Dry_farmers and Temp variables (Table 2). More likely, the concurrence of a higher proportion of Dry-land farmers (riskier activity) and higher NDVI (harboring more chiggers) may have led to occurrence higher incidence of scrub typhus in mountainous Taiwan. When examined in the spatial context, chigger survival in Taiwan may not be as limited by the mild subtropical climate as by the absence of a suitable vegetative habitat, thus explaining the negative relationship between temperature and SIR.

Spatial variation in chigger abundance could not be well represented by *C. attenuata*, *S. murinus*, *M. caroli*, and *M. musculus* because these species were only lightly infested with chiggers even in scrub typhus-endemic regions (Kuo et al., 2011). That *A. agrarius*, *B. indica*, and *R. losea* harbored many more chiggers in high-SIR districts nevertheless revealed that spatial variation in SIR could be partially attributed to differences in vector abundance. This supports previous findings that the prevalence of chiggers in small mammal hosts is higher in eastern Taiwan than elsewhere on the island (Hasegawa et al., 1990). The higher OT seropositivity rates in rodents in high-SIR regions may be due to infestation with a greater number of chiggers, chiggers that were more frequently infected with OT, or both. Further investigation of OT infection rates in chiggers should help answer this question.

This population-level study represents an important first step towards understanding the spatial clustering of scrub typhus in Taiwan and the potential underlying causes. Additional individual-level, case-control studies on risk factors associated with scrub typhus should be conducted to support these hypotheses. Studies that address the direct influence of environmental factors on chigger abundance are also needed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2011.05.018.

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