

Cascading effect of economic globalization on human risks of scrub typhus and tick-borne rickettsial diseases

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Abstract. The increase in global travel and trade has facilitated the dissemination of disease vectors. Globalization can also indirectly affect vector-borne diseases through the liberalization of cross-border trade, which has far-reaching, worldwide effects on agricultural practices and may in turn influence vectors through the modification of the ecological landscape. While the cascading effect of economic globalization on vector-borne diseases, sometimes acting synergistically with regional agricultural policy, could be substantial and have significant economic, agricultural, and public health implications, research into this remains very limited. We evaluated how abandonment of rice paddies in Taiwan after joining the World Trade Organization, along with periodic plowing, an agricultural policy to reduce farm pests in abandoned fields can unexpectedly influence risks to diseases transmitted by ticks and chiggers (larval trombiculid mites), which we collected from their small-mammal hosts. Sampling was limited to abandoned (fallow) and plowed fields due to the challenge of trapping small mammals in flooded rice paddies. Striped field mice (*Apodemus agrarius*) are the main hosts for both vectors. They harbored six times more ticks and three times more chiggers in fallow than in plowed plots. The proportion of ticks infected with *Rickettsia* spp. (etiologic agent of spotted fever) was three times higher in fallow plots, while that of *Orientia tsutsugamushi* (scrub typhus) in chiggers was similar in both treatments. Fallow plots had more ground cover and higher vegetation than plowed ones. Moreover, ticks and chiggers in both field types were dominated by species known to infest humans. Because ticks and chiggers should exhibit very low survival in flooded rice paddies, we propose that farm abandonment in Taiwan, driven by globalization, may have inadvertently led to increased risks of spotted fever and scrub typhus. However, periodic plowing can unintentionally mitigate vector burdens. Economic globalization can have unexpected consequences on disease risk through modification of the agricultural landscape, but the outcome may also be influenced by agricultural policies, calling for further research on vector-borne diseases and their control from broader perspectives.

Key words: agriculture; agricultural policy; economic globalization; *Orientia tsutsugamushi*; rice; *Rickettsia* spp.; Taiwan rice paddies; tick; trombiculid mite; vector-borne diseases; World Trade Organization.

INTRODUCTION

Considerable increase in global travel and trade has facilitated the dissemination of disease vectors, leading to rapid spread of vector-borne diseases around the world (Tatem et al. 2006, Randolph and Rogers 2010). A Middle East origin of an outbreak of mosquito-borne West Nile virus in the northeastern United States suggests a global introduction (Lanciotti et al. 1999). Similarly, continual movement of people and transport of competent mosquito vectors may explain the rapid spread of Chikungunya fever to Indian Ocean islands, India, and Europe within three years of the 2004

outbreak in Kenya (Charrel et al. 2007, Staples et al. 2009).

Globalization also may indirectly affect human risks to vector-borne diseases through liberalization of cross-border trade in agricultural commodities. Most disease vectors are arthropods that may readily integrate into the benign environmental conditions of many agricultural landscapes (Sutherst 2004, Chaves and Koenraadt 2010). For example, agricultural practices have enhanced vectors of leishmaniasis in Tunisia and Israel (Ben Salah et al. 2000, Wasserberg et al. 2003) and vectors of malaria in Belize (Pope et al. 2005), Ethiopia (Ye-Ebiyo et al. 2000), and Asia (Service 1989, Keiser et al. 2005), although they may also suppress tsetse flies (*Glossina* spp.) in Africa (Reid et al. 2000) and vectors of malaria after forests were replaced by tapioca plantations in Thailand (Service 1991). At the same time, global trade, enhanced by liberal trade agreements, has far-reaching effects on the extent and intensity of

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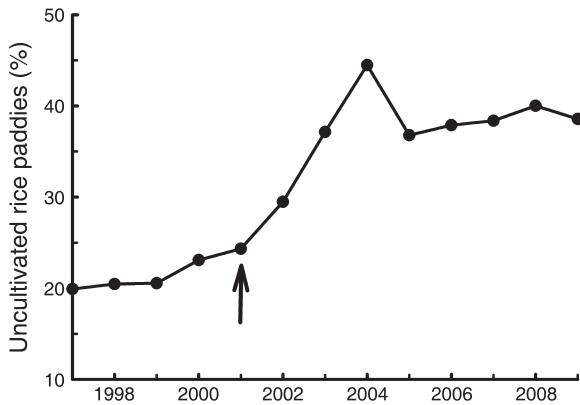


FIG. 1. Annual variation (1997–2009) in percentage of rice paddies left fallow (i.e., not cultivated) in Taiwan. Taiwan joined the World Trade Organization at the end of 2001 (arrow). The data are from the Agricultural and Food Agency, Taiwan Council of Agriculture (in Chinese): <http://www.afa.gov.tw/>

agricultural activities worldwide and has dramatically influenced the ecological landscape, even in remote localities (Archer et al. 2008, Lambin and Meyfroidt 2011, Neumann et al. 2011). This is especially true for developing countries, where lower production costs, along with the lack of political power to repel trade liberalization (Keyder and Yenil 2011), can often lead to intensified agricultural operations. Additionally, these countries often are situated in tropical areas with heavy burdens of vector-borne diseases, where deforestation, frontier colonization, and construction of irrigation systems may facilitate increased contact with disease vectors (Service 1991, Briceño-León 2007). Emergence of Chagas disease in the Amazon has been associated with extensive ranching and soybean cultivation that has been driven by global demand (Briceño-León 2007), and mite-borne scrub typhus has become more prevalent in Malaysia following extensive development of oil palm plantations (Abdullah and Hezri 2008, Kwa 2008).

On the other hand, globalization may promote abandonment of farmlands where commodities are no longer price competitive, and this also may influence the distribution of disease vectors. Reduced rice cultivation in France during the 1960s due to competition from Italy and a lack of governmental subsidies led to the decline of mosquitoes (Poncon et al. 2007). Abandonment of farmlands also may facilitate emergence of vector-borne diseases that previously were suppressed by farming activities. The emergence of Lyme disease in the northeastern United States was partially attributed to forest regeneration after agricultural fields were abandoned in the mid-19th century (Barbour and Fish 1993), in part a result of domestic competition from more fertile lands in the western United States (Hall et al. 2002). While the cascading effect of economic globalization on risks to vector-borne diseases, through economic incentives on agricultural landscape, can be

substantial, related studies remain very limited. Similarly, little effort has assessed how globalization may act synergistically with regional agricultural policy in response to global trade (e.g., governmental subsidies) to indirectly influence human risks to vector-borne diseases. Such investigation, nevertheless, can have very important implications for formation of policy that can benefit both food security and public health.

Taiwan's agricultural landscapes have been dramatically altered since it joined the World Trade Organization (WTO) at the end of 2001. WTO-mandated removal of trade barriers exposed Taiwan's agricultural products, including rice, to keen foreign competition. In response to higher production costs relative to other developing countries, a paucity of labor forces in rural areas, and financial incentives from the government, many Taiwanese rice paddies have been abandoned (Huang et al. 2009). Whereas about 20% of paddies were not cultivated before 2001, this percentage increased substantially to nearly 45% by the end of 2004, and finally stabilized at around 40% (Council of Agriculture, Taiwan 2011; Fig. 1). Moreover, Taiwanese rice fields are highly fragmented, and average only 1.14 ha in area (Huang et al. 2002), so cultivated farms frequently are adjacent to abandoned fields. Farmers are subsidized by the government to plow fallow paddies twice yearly; lacking this, these abandoned rice fields have the potential to be a source of agricultural pests to neighboring planted crops. However, plowing is not enforced, so while some uncultivated paddies are plowed periodically, others are completely abandoned.

Hard ticks (Ixodidae) and trombiculid mites (Trombiculidae), which can transmit tick-borne spotted fever group (SFG) rickettsiae (*Rickettsia* spp.) and scrub typhus (*Orientia tsutsugamushi*, OT), respectively, to humans in Taiwan, are two disease vectors that could potentially be affected by agricultural practices. Tick-borne SFG rickettsiae are emerging worldwide (Parola et al. 2005), including Taiwan (Tsui et al. 2007, Tsai et al. 2008), and scrub typhus is currently the most common rickettsial disease in Taiwan (Tsai et al. 2008). SFG rickettsiae can be transmitted in ticks both transstadially (from larva to nymph to adult) and transovarially (from adult to progeny) (Raoult and Roux 1997), with some filial and transstadial transmission efficiency reaching 100% (Macaluso et al. 2002, Pacheco et al. 2011). Consequently, all three parasitic life stages (larvae, nymphs, adults) have the potential to be infective and to transmit SFG rickettsiae to humans. Ticks are reservoirs of SFG rickettsiae; it is less clear whether their vertebrate hosts, mostly mammals, also serve as reservoirs of these pathogens (Raoult and Roux 1997, Parola and Raoult 2001).

The life cycle of trombiculid mites includes seven stages: egg, deutonymph, larva (chigger), protonymph, deutonymph, tritonymph, and adult. Only chiggers are parasitic. Nymphs and adults are free-living in the soil, feeding mainly on the eggs and larvae of arthropods

(Kawamura et al. 1995). *Leptotrombidium* chiggers are the primary vectors for OT, and murine rodents are the predominant hosts (Traub and Wisseman 1974, Kawamura et al. 1995). Humans are accidental hosts, and become infected with scrub typhus when bitten by infected chiggers. Because only chiggers are parasitic and they feed only once, both transstadial and transovarial transmission must occur for unfed chiggers to be infective (Frances et al. 2001). Indeed, extremely high filial transmission efficiency has been documented for some chigger species (Frances et al. 2001, Phasomkusolsil et al. 2009). Trombiculid mites are the only reservoirs of OT, while vertebrate hosts provide chiggers with food resources but play little role in transmitting OT (Kawamura et al. 1995). We defined hosts solely as providers of food resources, with no implication of any role as disease reservoirs.

Flooding and low relative humidity provide poor environments for both ticks (Stafford 1994, Bunnell et al. 2003, Manangan et al. 2007) and chiggers (Traub and Wisseman 1974, Muul et al. 1977, Kawamura et al. 1995). Because rice cultivation in this region normally involves flooding (Wang and Apthorpe 1974, Piper 1993), both disease vectors likely are suppressed in rice paddies. Abandonment of rice fields may lead to an increase in ticks and chiggers. Growth of vegetation could also favor demographic expansion by their small-mammal hosts. On the other hand, plowing of fallow fields removes vegetation and exposes soils to desiccation, leading to lower soil humidity, so we expect a lower number of ticks and chiggers after fallow fields are plowed.

The purpose of this study was to evaluate whether globalization-induced abandonment of rice paddies in Taiwan has been associated with increased risk of tick- and chigger-borne diseases, and whether periodic plowing could reduce this risk. The risk of infection is a function of the abundance and infective rates of questing vectors. Unfortunately, the inefficiency of sampling free-living ticks and chiggers prevented us from directly quantifying their abundance. Instead, we compared abundance and pathogen infectivity of ticks and chiggers recovered from rodents captured through extensive trapping efforts. Because of practical challenges associated with trapping rodents in flooded rice paddies, we focused our efforts on abandoned ("fallow") and plowed plots. We do not consider this to be a limitation to this study because we are primarily interested in the response by rodents, ticks, and chiggers to habitat management, and available information strongly suggests that very few or no questing hard ticks and chiggers occur in submerged rice paddies (we address this further in the *Discussion*). We also characterize the association between host loads of ticks and chiggers as functions of microhabitat and rodent abundance, two factors likely to determine vector abundance (Traub and Wisseman 1974, Muul et al.

1977, Schulze et al. 2002, Sinski et al. 2006, Medlock et al. 2008).

We test the following explicit hypotheses: (1) plowing of abandoned fields will reduce the risks to tick- and chigger-borne diseases (specifically, SFG rickettsiae and scrub typhus) relative to unplowed fields; (2) abundance of ticks and chiggers will be determined more by environmental factors than by rodent abundance because both vectors spend the majority of their life off the hosts. Additionally, we investigate whether abandonment of rice paddies would likely increase the risk to SFG rickettsiae and scrub typhus. While field logistics dictate that this research is limited in geographical scale, it nevertheless provides a compelling case study on the effect of broadscale political and economic policies on disease risks, through impact on local agricultural practices and cascading influences on vectors of diseases.

METHODS

Study area

We studied agricultural fields in the lowlands of Hualien County, Taiwan, where agriculture dominates land use, and villages are interspersed among fields. Farms typically are fragmented, with planted, plowed, and fallow rice fields adjacent to each other. Cultivated rice lands were flooded (Fig. 2). Fallow plots generally were dominated by perennial Japanese silvergrass (*Miscanthus floridulus*) and an invasive evergreen (*Leucaena glauca*). Plowed plots are rapidly colonized by the invasive herb *Bidens pilosa* var. *radiata*, and vegetation generally reaches 0.5 m high within 6 months, when plowing is repeated (Fig. 2).

Although Hualien is one of Taiwan's least populated counties, it had the country's highest cumulative human incidence of scrub typhus between 1998 and 2007 (524 cases, 15.4% of total incidence in Taiwan; Centers for Disease Control, Taiwan 2008) except for a single offshore islet (Kinmen Island, 558 cases), and this disease has been endemic there for ≥ 95 years (Hatori 1919). The geographical distribution of human cases of tick-borne spotted fever in Taiwan is not known because this is not a notifiable disease, but an unrecognized strain of spotted fever (*Rickettsia* sp.) was recovered from ticks in Hualien (Tsui et al. 2007).

Small-mammal trapping and collection of ticks and chiggers

We surveyed small mammals in plowed plots and plots that were left fallow between August 2007 and March 2008. Sampling efforts were alternated by agricultural treatment (plowed, fallow) to control for temporal effects. Mobility is very limited in ticks (< 10 m; Falco and Fish 1991) and chiggers (< 45 cm; Philip et al. 1949). Moreover, because two of the largest host species (the greater bandicoot rat, *Bandicota indica* and the lesser rice field rat, *Rattus losea*) rarely move > 500 m (Wang and Wang 2001), study plots were separated by



FIG. 2. Illustrations of different forms of land use in Taiwan: (a) flooded rice paddy; (b) fallow plot; (c) plowed plot just after the plowing, illustrating the exposed surface soil; and (d) plowed plot with vegetative regrowth about six months after plowing.

at least 500 m to reduce potential spatial dependence; because these two rodents likely have larger home ranges than smaller species (McNab 1963), we believe this distance is sufficient to ensure independence. Because we intended to sample as many plots as possible, we characterized rodent density as number of individuals captured per trap night instead of estimating absolute density with the mark–recapture method. In each plot, we deployed three parallel lines of 10 Sherman traps ($26.5 \times 10 \times 8.5$ cm) at 10-m intervals, and three handmade live traps ($27 \times 16 \times 13$ cm) at 30-m intervals. Handmade traps were used to target the larger but less abundant species such as *B. indica*. Adjacent transect lines also were separated by 10 m. Traps were opened and baited in the evening and checked for captures early in the morning. Each plot was surveyed once and each trapping bout lasted for three consecutive nights; hence, each site received the same sampling effort (117 trap nights).

Trapped rodents were transferred to a clean nylon mesh bag (bags were carefully examined to ensure that no arthropod vectors remained from earlier captures), anesthetized with Zoletil 50 (Virbac, Carros, France), and examined for sex and reproductive status. We recorded body mass with a spring scale (accuracy 0.5 g) and standard external measurements (length of head

plus body, tail, ear, and hind foot), and thoroughly examined animals for ectoparasites by carefully combing their fur. Ticks were directly preserved in 70% ethanol. Skin with attached chiggers was detached with minimum injury to the animals and preserved in vials; chiggers were transferred to 70% ethanol after two days. Ticks and chiggers recovered from animals were counted individually. Rodents with fur clips were released outside the study areas (except for the exotic Pacific rat, *Rattus exulans* and rice field rat, *R. argentiventer*, which were humanely euthanized) instead of at the trapping sites as part of a related investigation of colonization success by native vs. exotic rodent species (C. C. Kuo, J. L. Huang, and H. C. Wang, *unpublished data*). All procedures were approved by the University of California, Davis Animal Use and Care Administrative Advisory Committee, met guidelines recommended by the American Society of Mammalogists (Sikes et al. 2011), and fulfilled Taiwanese legislative requirements.

Tick and chigger identification

We identified a minimum of one-fifth of chiggers recovered from each host individual. Chiggers were soaked in deionized water and then slide-mounted in Berlese fluids (Asco Laboratories, Manchester, UK), and examined under a light microscope (Tokyo,

TABLE 1. Primers for polymerase chain reaction (PCR) specific for 17-kDa antigen in *Rickettsia* spp. (spotted fever) and 56-kDa antigen in *Orientia tsutsugamushi* (scrub typhus).

Primer	Sequence (5' → 3')
Real-time PCR assay for <i>Rickettsia</i> spp. in ticks	
17kDa-142F	GGT ATG AAT AAA CAA GGT ACA GGA AC
17kDa-447R	ATA TTG ACC AGT GCT ATT TCT ATA AG
17kDa-139F	GGG TGG TAT GAA CAA ACA AGG GAC TG
17kDa-378R	CGC CAT TCT ATG TTA CTA CCG CTA GG
17kDa-133F	TGG TCA GAG TGG TAT GAA CAA ACA AG
Nested PCR assay for <i>Orientia tsutsugamushi</i> in chiggers	
1st stage	
56kDa-1F	AGA ATC TGC TCG CTT GGA TCC A
56kDa-1R	ACC CTA TAG TCA ATA CCA GCA CAA
2nd stage	
56kDa-2F	GAG CAG AGA TAG GTG TTA TGT A
56kDa-2R	TAT TCA TTA TAG TAG GCT GA

Olympus, Japan) for identification with published keys (Wang and Yu 1992, Li et al. 1997). Ticks were keyed directly under a stereomicroscope (Leica, Heerbrugg, Switzerland) and identified with published keys (Teng and Jiang 1991). Uncertain tick species were confirmed by comparing 12S rDNA sequence with known species (Beati and Keirans 2001).

Pathogen detection in ticks and chiggers

Most ticks recovered from rodents were larval and nymphal *Rhipicephalus haemaphysaloides* (see *Results*), which were analyzed with real-time polymerase chain reaction (PCR) specific for the 17-kDa antigen in *Rickettsia* spp. A single tick was selected from most rodents (from three rodents we selected two ticks each to increase sample size), with rodents chosen to best include all plots, host sex, and full gradients of tick loads within each field type (plowed and fallow). Bacterial DNA was extracted from ticks and purified with QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The PCR amplification reaction mixture contained 10 μ L of 2 \times QuantiTect SYBR Green PCR Master Mix (QIAGEN), 2 μ L DNA templates, 6 μ L RNase-free water, and 2 μ L each of the 5 μ mol/L primers (see Table 1 for primers used). DNA fragments were amplified with one cycle of 15 min at 95°C, 45 cycles of 15 s at 94°C, 30 s at 55°C, 38 s at 72°C, one cycle of 1 min at 95°C, and 45 cycles of 30 s at 65°C. The PCR reaction was implemented and DNA products analyzed in a quantitative PCR system (Mx3005P; Stratagene, La Jolla, California, USA). A positive control of *R. rickettsii* and *R. typhi* DNA was included for each test.

Orientia tsutsugamushi (OT) was assayed in chiggers of the genus *Leptotrombidium* with nested PCR. To retrieve sufficient DNA for analysis, nested PCR was performed on pools of 30 chiggers. Chiggers were grouped into genera because further classification (i.e., to species) would require the use of Berlese fluids, which destroys DNA material. Chiggers of the same pool were recovered from the same host individual, selected using

the same criterion as ticks. We followed Kawamori et al. (1993) in the detection of OT in chiggers, which targeted a well-conserved DNA corresponding to 56-kDa type-specific antigen located on the OT outer membrane. Chiggers were ground in 0.1-mL SPG buffer (3.0 mmol/L KH_2PO_4 , 7.2 mmol/L K_2HPO_4 , 40 mmol/L L-glutamic acid, and 218 mmol/L sucrose in distilled water, pH 7.0), then centrifuged at 1138×10^2 m/s² for 5 min. The resulting pellets were suspended in 50 μ L 10 mmol/L Tris-HCl buffer at pH 8.3 holding 0.1 mg proteinase K, 0.5% Nonidet P-40, 0.5% Tween 20, incubated at 56°C for one night, and heated to 95°C for 10 min. The products were used for PCR reaction. The first stage PCR amplification reaction mixture contained 35.5 μ L deionized water, 5 μ L of 10 \times PCR buffer, 2 μ L of 5 mmol/L dNTPs, 3 μ L of 25 mmol/L MgCl_2 , 3 μ L DNA templates, 0.5 μ L *Taq* enzyme, and 1 μ L each of the 5 μ mol/L primers (Table 1). DNA fragments were amplified with one cycle of 3 min at 94°C, 40 cycles of 45 s at 94°C, 45 s at 50°C, 45 s at 72°C, and one cycle of 7 min at 72°C. The reaction mixture of the second stage PCR was the same as before except 1 μ L each of the 20- μ mol/L primers (Table 1) were added and 3 μ L products from the previous stage as the DNA templates. Fragments were amplified with one cycle of 3 min at 94°C, 25 cycles of 45 s at 94°C, 45 s at 50°C, 45 s at 72°C, and one cycle of 7 min at 72°C. The positive PCR products were separated by electrophoresis in 1.5% agarose gels, stained with ethidium bromide, and identified under UV fluorescence. Laboratory Karp and Gilliam strains and deionized water were used as positive and negative controls, respectively.

Microhabitat measurements

We recorded four microhabitat characters in a 1-m² quadrat established at each trapping station. These included vegetative height (centimeters) and ground cover (percent) for plant litter and both understory (≤ 2 m) and overstory vegetation (> 2 m). Crown cover was not measured because trees were uncommon in our study fields (see Fig. 2). Vegetative height was repre-

sented by the mean of 10 randomly sampled points for each trap station. We used a vertical rule to measure vegetative height. Ground cover for the litter, understory layer, and overstory layer was estimated by placing a 1×1 m sampling quadrat (with 10 equidistant marks on each side to facilitate visual separation of 100 subquadrats) on the ground. All habitat measurement was implemented within one week of the trapping bout.

Analysis

Because all plots were spatially separate, and each plot was sampled only once, we treated plots as independent measures, except where stated otherwise. We used nested ANOVA to compare tick and chigger loads between the two field types (fallow and plowed) because tick and chigger loads within the same plot may respond similarly to extrinsic influences, but we did not take into account host characteristics that might influence vector loads (e.g., sex, mass) because of no obvious difference in host characteristics between the two field types. We pooled larval and nymphal ticks for analysis because both stages can transmit *Rickettsia* spp. Because assumptions of normality and homogeneity of variance could not be met even after transformation, we used ranked input rather than raw data, making this a nonparametric analysis. Tick and chigger loads were ranked separately across all plots, and rank was then treated as the dependent variable nested within plots. Plots were then treated as replicates within field type as in parametric nested ANOVA (Conover and Iman 1981). We also provide mean values of original (raw) vector loads for each field type to facilitate comparisons; for this, we calculated mean tick or chigger loads for all rodents from a given plot, and then calculated the mean across plots within each field type.

Difference in prevalence of tick and chigger infestation (number infested/total rodents) between fallow and plowed plots was compared with a *t* test when both normality and homogeneity of variance can be confirmed (with Shapiro-Wilk and Levene tests, respectively), and with a nonparametric Mann-Whitney *U* test otherwise.

We tested differences in pathogen infective rates in both ticks and chiggers between fallow and plowed plots using logistic regression. We treated individual samples (ticks and chiggers) as independent replicates, and pooled our data across plots within each field type because the number of vectors for disease assay was limited (i.e., analytical sample size was $n = 2$ field types, each data value deemed independent). Such pooling should not be a problem because vectors selected for pathogen assay were taken from host rodents chosen to evenly sample all plots.

We also applied logistic regression to compare available microhabitat and trapping success of rodents between fallow and plowed plots. Microhabitat of a given plot was represented by the average of all trap stations (39 trap stations for each plot). To avoid

multicollinearity, explanatory variables with a variance inflation factor >10 were excluded from further analyses. Outliers were identified with a deviance residual value >2 , and goodness of fit of the logistic model was assessed with a Hosmer-Lemeshow test.

Finally, because heteroscedasticity in our data could not be eliminated even after transformation, we applied a bootstrap (9999 iterations, with replacement) to estimate the 95% confidence intervals for regression coefficients relating vector loads to both microhabitat and trapping success of rodents. Vector loads may be determined by environmental factors other than microhabitat (e.g., seasons; Brunner and Ostfeld 2008), and inclusion of these environmental factors would confound the real influence of microhabitat. Consequently we corrected for the effects of plowing (see *Results*) and season (Kuo et al. 2011b) on vector loads by calculating the mean tick and chigger loads for each combination of treatment (fallow or plowed) and season (three seasons each based on significant difference in tick and chigger loads on striped field mice *Apodemus agrarius*; Kuo et al. 2011b). Our dependent variable (tick or chigger load) was calculated as the difference from the mean for each treatment \times season combination (six combinations each for ticks and chiggers). Larval and nymphal ticks were analyzed separately because they may be differentially influenced by host density (Brunner and Ostfeld 2008). All procedures were implemented in JMP 8 (SAS Institute, Cary, North Carolina, USA) or Stata 11 (StataCorp, College Station, Texas, USA).

RESULTS

Small-mammal trapping

We captured 10 species of small mammals, including 764 individuals of nine species in 21 plowed plots (2457 trap-nights), and 521 individuals of eight species in 20 fallow plots (2340 trap-nights) (Table 2). The most abundant species in both treatments were *A. agrarius* (37.4%), Ryukyu mouse (*Mus caroli*; 30.7%), and house mice (*M. musculus*; 17.3%) (Table 2). *A. agrarius* hosted the greatest percentage of ticks (77.6%), followed by *R. losea* (19.7%); hence, these two host species accounted for $>97\%$ of total ticks. Similarly, *A. agrarius* hosted the greatest percentage of chiggers (46.5%), although *R. losea* was only slightly lower (42.5%). *B. indica* also supported 10.9% of the chiggers retrieved. More than 99% of chiggers were recovered from these three species. In contrast, the abundant *M. caroli* and *M. musculus* were only infrequently infected with both ticks and chiggers (Table 2).

We selected *A. agrarius* for comparison among plots because of its high mean rate of infestation with both ticks and chiggers, its abundance in most plots, and its high individual variability in tick and chigger loads (0–575 chiggers; 0–109 ticks). We discarded eight plots with less than five *A. agrarius* captures. Thus, we analyzed captures of 460 individual *A. agrarius* in 33 plots (251

TABLE 2. Abundance of small mammals, hard ticks, and chiggers (larval trombiculid mites) recovered in plowed and fallow plots (21 and 20 plots, respectively) between August 2007 and March 2008 in Hualien County, Taiwan.

Species	Small mammals		Ticks		Chiggers	
	Plowed	Fallow	Plowed	Fallow	Plowed	Fallow
<i>Apodemus agrarius</i>	262	218	128	926	13 568	28 352
<i>Bandicota indica</i>	20	11	15	14	3 062	6 800
<i>Crocidura attenuata</i>	6	6	0	1	0	3
<i>Crocidura suaveolens</i>	0	2	0	1	0	5
<i>Mus caroli</i>	276	119	0	2	0	8
<i>Mus musculus</i>	119	103	0	2	1	0
<i>Rattus argentiventer</i>	1	0	0	0	47	0
<i>Rattus exulans</i>	21	0	0	0	26	0
<i>Rattus losea</i>	30	42	45	223	12 527	25 750
<i>Suncus murinus</i>	29	20	0	2	0	0
Total	764	521	188	1 171	29 231	60 918

captures in 17 plowed plots, 209 captures in 16 fallow plots).

Species composition and loads of ticks and chiggers in plowed vs. fallow plots

We examined a total of 1042 ticks collected from *A. agrarius*. Larval and nymphal *Rhipicephalus haemaphysaloides* dominated in both plowed (99.2%) and fallow (99.8%) plots (Table 3). The remaining ticks belonged to *Ixodes granulatus* and *Haemaphysalis* sp. Among 10 426 chiggers identified (25.6% of total chiggers recovered from *A. agrarius*), *Leptotrombidium imphalum* was the most common species (plowed, 90.8%; fallow, 80.9%), followed by *L. deliense* (plowed, 7.5%; fallow, 18.5%; Table 3). Fewer percentages of chiggers belonged to the *Gahrlipeia* spp. and other *Leptotrombidium* species.

Because ticks recovered from *A. agrarius* were dominated by larvae and nymphs, both capable of transmitting *Rickettsia* spp., they were pooled together for analysis. Mean tick loads of *A. agrarius* (including both infested and noninfested individuals) in fallow plots were more than six times those in plowed ones (original values: 3.9 vs. 0.6; $F_{1,31} = 15.0$, $P < 0.001$,

nested ANOVA using ranked data; Fig. 3a). This was primarily a reflection of greater tick infestation rates in fallow plots (64.3% vs. 21.0%; t test, $t = 4.58$, $df = 31$, $P < 0.001$), but may also reflect a trend for greater tick loads (including tick-infested *A. agrarius* only) in fallow plots (5.0 vs. 2.3 individuals; $F_{1,27} = 1.5$, $P > 0.05$). Mean chigger loads in fallow plots were nearly three times those of plowed ones (145.9 vs. 50.9 individuals; $F_{1,31} = 11.4$, $P < 0.005$; Fig. 3b). Unlike patterns for ticks, the prevalence of chigger infestation was similar in both treatments (99.5% vs. 87.4%; Mann-Whitney U test, $U = 108$, $P > 0.05$), but chigger loads were higher in fallow than in plowed plot (146.9 vs. 52.4; $F_{1,31} = 12.9$, $P < 0.005$; chigger-infested individuals only).

Pathogen prevalence in plowed vs. fallow plots

We assayed 120 ticks from 117 *A. agrarius* individuals (25.4% of *A. agrarius* captures) for *Rickettsia* infection. The percentage of infected *Rickettsia* spp. in larval and nymphal *R. haemaphysaloides* ticks (57 and 63 ticks assayed for plowed and fallow, respectively) was about three times higher in fallow than in plowed plots (39.7% vs. 14.0%; logistic regression, likelihood ratio [LR] $\chi^2 =$

TABLE 3. Species composition of hard ticks and chiggers (larval trombiculid mites) recovered from *Apodemus agrarius* (striped field mice) in plowed and fallow plots (17 and 16 plots, respectively) between August 2007 and March 2008 in Hualien County, Taiwan.

Species	Plowed		Fallow	
	<i>n</i>	%	<i>n</i>	%
Tick				
<i>Rhipicephalus haemaphysaloides</i>	125	99.2	914	99.8
Larva	88	70.4	698	76.4
Nymph	37	29.6	216	23.6
<i>Ixodes granulatus</i>	0	0	2	0.2
<i>Haemaphysalis</i> sp.	1	0.8	0	0
Total	126		916	
Chigger				
<i>Leptotrombidium imphalum</i>	3408	90.8	5400	80.9
<i>Leptotrombidium deliense</i>	282	7.5	1234	18.5
<i>Leptotrombidium</i> spp.	62	1.7	24	0.4
<i>Gahrlipeia</i> spp.	2	<0.1	14	0.2
Total	3754		6672	

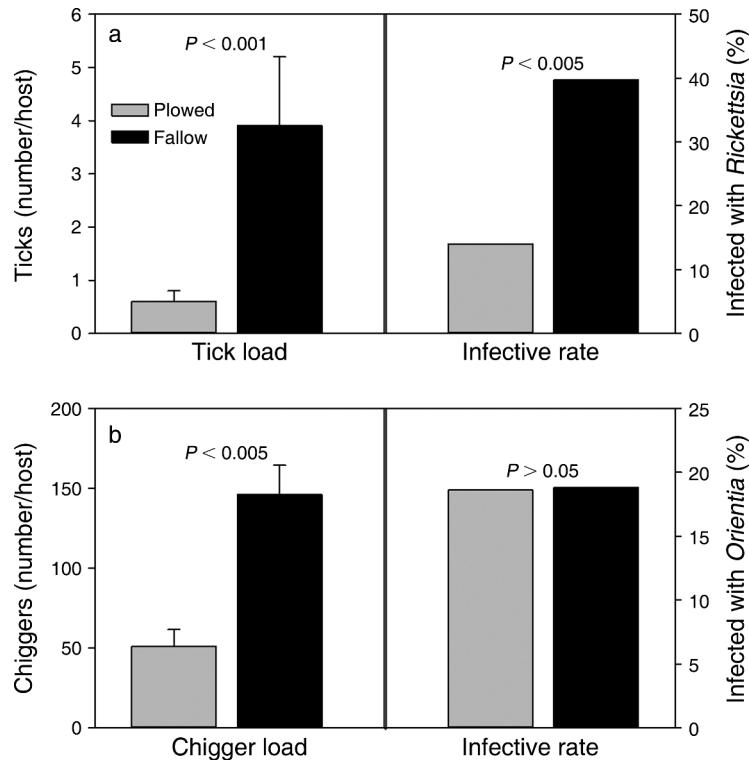


FIG. 3. Comparison of abundance of disease vectors and pathogen infective rate in vectors in plowed vs. fallow plots: (a) abundance and infective rate of *Rickettsia* spp. (spotted fever) in hard ticks recovered from the rodent *Apodemus agrarius* between August 2007 and March 2008 in Hualien County, Taiwan; and (b) abundance and infective rate of *Orientia tsutsugamushi* (scrub typhus) in chiggers (larval trombiculid mites). All data are presented as mean + SE.

10.3, $df = 1$, $P < 0.005$; Fig. 3a). Fallow plots had higher infective rates in both larvae (41.9% vs. 13.8%; LR $\chi^2 = 6.1$, $df = 1$, $P < 0.05$; 31 and 29 ticks for fallow and plowed, respectively) and nymphs (37.5% vs. 14.3%; LR $\chi^2 = 4.3$, $df = 1$, $P < 0.05$; 32 and 28 ticks assayed). A total of 139 pools of chiggers from 139 *A. agrarius* individuals (30.2% of *A. agrarius* captures) were assayed for OT infection. Infective rate of OT in *Leptotrombidium* chiggers (70 and 69 pools of chiggers assayed for plowed and fallow, respectively) was similar in plowed and fallow plots (18.6% vs. 18.8%; LR $\chi^2 < 0.01$, $df = 1$, $P > 0.05$; Fig. 3b).

Microhabitat and trapping success of rodents in plowed vs. fallow plots

We assessed trapping success for *A. agrarius*, *R. losea*, and *B. indica* because these species accounted for the majority of recovered ticks and chiggers. The variance inflation factors for all seven explanatory variables (four microhabitat variables and trapping success for three rodent species) were all < 2 , indicating a low degree of multicollinearity. Deviance residuals for all 33 replicates were also < 2 , revealing a lack of important outliers in the logistic regression model. Thus, we included all variables and samples in the analysis.

Litter cover and vegetative height were greater in fallow than in plowed plots (litter, mean: 44.4% vs. 12.3%, LR $\chi^2 = 15.2$, $df = 1$, $P < 0.0001$; height, 51.7 vs. 48.6 cm, LR $\chi^2 = 5.7$, $df = 1$, $P < 0.05$, respectively), but trapping success for *B. indica*, while generally low across all sites, was higher in plowed than in fallow plots (0.005 vs. 0.009 individuals/trap-nights, respectively; LR $\chi^2 = 7.1$, $df = 1$, $P < 0.01$). Other microhabitat variables and trapping success of both *A. agrarius* and *R. losea* were similar in fallow and plowed plots (all $P > 0.05$; Table 4). The nonsignificance of the Hosmer-Lemeshow goodness-of-fit test ($\chi^2 = 0.56$, $P = 0.99$) indicated a good fit of the logistic model.

Association between vector loads and microhabitat and trapping success of rodents

Bootstrap analysis revealed no significant association between tick loads on *A. agrarius* (after correction for seasonal and plowing effects) and trapping success of *A. agrarius*, *R. losea*, *B. indica*, or with any of the four microhabitat variables (Appendix). Similarly, we found no relationship between loads of larval ticks, nymphal ticks, or chiggers on *A. agrarius* and the seven explanatory variables (Appendix).

TABLE 4. Logistic regression analyses of difference in microhabitat and trapping success of rodents (individuals/trap night) between plowed and fallow plots (17 and 16 plots, respectively) in Hualien County, Taiwan.

Variable†	Mean value		Regression coefficient‡	LR χ^2 §	df	P
	Fallow	Plowed				
<i>Apodemus agrarius</i>	0.11	0.13	0.20	1.8	1	0.18
<i>Rattus losea</i>	0.019	0.015	0.56	0.4	1	0.51
<i>Bandicota indica</i>	0.005	0.009	-5.69	7.1	1	<0.01
Vegetative height (cm)	51.7	48.6	0.27	5.7	1	0.02
Plant litter (%)	44.4	12.3	3.87	15.2	1	<0.0001
Understory layer (%)	79.7	88.6	-2.24	3.5	1	0.06
Overstory layer (%)	38.3	1.2	21.19	0.3	1	0.58

† See *Methods: Microhabitat measurements* for the definition of variables.

‡ Fallow relative to plowed plots.

§ Likelihood ratio chi-square.

DISCUSSION

Fallow plots in our study had taller vegetation and more litter cover but lower numbers of *B. indica* (based on trap success) than did plowed plots. In contrast, other microhabitat variables were similar across treatment types, as were numbers of other common species, *A. agrarius* and *R. losea*. Notably, ticks were six times more abundant on *A. agrarius* in fallow than in plowed plots, reflecting the higher prevalence of tick infestation rather than higher tick loads among infested rodents in fallow plots; those *A. agrarius* in plowed plots that harbored ticks had as many as did individuals in fallow plots. Similarly, chiggers were three times more abundant in fallow than in plowed plots; however, while prevalence was similar between the two treatments, chigger loads among infested *A. agrarius* were higher in fallow than in plowed plots. Because the abundance of *A. agrarius* was similar in both field types, differences in vector loads were unlikely to be caused by differences in *A. agrarius* density or by different spacing behaviors in the two field types, as corroborated by the lack of association between vector loads and trapping success of *A. agrarius*. Moreover, the dominant tick (mostly *R. haemaphysaloides*) and chigger (predominantly *L. imphalum* and *L. deliense*) species also infest humans (Kawamura et al. 1995, Tanskul et al. 1998; C. C. Kuo, *personal observation*). Finally, ticks in fallow plots were much more likely to be infected by SFG rickettsia than those in plowed plots, whereas chiggers were equally likely to be infected with *O. tsutsumagushi*. Because the risk of infection is a function of the abundance and infective rates of vectors, given the higher vector abundance or/and higher infective rates of vectors in fallow plots, these data overwhelmingly support our hypothesis that the threat of disease is greater in fallow than in plowed plots.

In our study ticks and chiggers recovered from *A. agrarius* had higher or similar pathogen infectivity (respectively) in fallow than in plowed fields. Vectors may, nevertheless, acquire pathogens more efficiently from some host species than others (Ostfeld and Keesing 2000, LoGiudice et al. 2003), suggesting that infectivity

in questing ticks or chiggers could be determined by hosts other than *A. agrarius*. However, this is unlikely to be the case here because transovarial transmission efficiency of OT and SFG rickettsiae is very high (Frances et al. 2001, Macaluso et al. 2002, Phasomkulsil et al. 2009, Pacheco et al. 2011); trombiculid mites are the only reservoirs of OT (Kawamura et al. 1995) and vertebrates play at most an auxiliary role in inoculating *Rickettsia* to ticks (Raoult and Roux 1997). This is in contrast to other pathogens in which transovarial transmission rarely occurs and vertebrates are necessary for infecting vectors. For example, *Borrelia burgdorferi*, with very low transovarial transmission efficiency, is vectored by hard ticks, and the white-footed mouse (*Peromyscus leucopus*) is mostly responsible for the occurrence of Lyme disease in the northeastern United States by maintaining and infecting larval ticks *B. burgdorferi* (LoGiudice et al. 2003). Infectivity in questing ticks and chiggers was therefore unlikely to be affected by other host species that were sheltered in plowed plots. On the other hand, the reason for the much higher infection rate of SFG rickettsia in ticks within fallow plots was unclear. Because infested *A. agrarius* had similar tick loads between the two field types, pathogen transmission through co-feeding on hosts may not explain the higher infective rate in fallow plots due to the similar degree of co-occurrence of ticks on the same host and thus similar chance of acquiring pathogen from other ticks. Whether higher infection rate of SFG rickettsia in fallow plots was related to their harboring larger tick populations (due to higher prevalence of similar infestation intensity) warrants further investigation.

Reduced numbers of ticks and chiggers in plowed plots were not caused by differences in host abundance; *A. agrarius* was similarly abundant on both treatments. Because lower relative humidity reduces survival rate in both ticks and chiggers (Traub and Wisseman 1974, Needham and Teel 1991, Stafford 1994, Randolph 2004), and plowed plots endure a period when surface soils are exposed directly to sunlight (Fig. 2c), presumably making soils drier, we suspect that lower soil humidity renders plowed fields less hospitable to ticks

and chiggers. In contrast, the greater litter cover and vegetative height in fallow plots may help retain soil moisture. For example, questing immature black-legged ticks (*Ixodes scapulari*) were largely suppressed following the removal of leaf litter, likely due to the accompanied reduction in soil moisture (Schulze et al. 1995). We attempted to compare soil relative humidity in plowed and fallow fields using remote data loggers, but these were destroyed by mechanistic plowing or typhoon-induced high rainfall, rendering comparison impossible. Further study could verify the association between reduced ticks and chiggers and lower soil humidity in plowed fields. On the other hand, nymphal and adult trombiculid mites are free-living and prey on soil arthropods; plowing likely influences the soil arthropod community, but whether this benefits or hinders them is not clear without further study.

In Taiwan, rice typically is produced two to three times annually (Wang and Apthorpe 1974), and rice paddies are flooded for at least three months during each cultivation cycle (Pan 2008). Ticks are terrestrial organisms, and avoid contact with water (Krober and Guerin 1999); once immersed in water, survival probability and oviposition capability decline dramatically, and most ticks cannot survive extended submersion (Sutherst 1971, Smith 1973, Paula et al. 2004, Adejinmi 2011, Fielden et al. 2011). For example, adult dog ticks (*Dermacentor variabilis*) can survive underwater for a maximum of 15 days (Fielden et al. 2011), and almost all larval tropical horse ticks (*Anocentor nitens*) die after immersion for five days (Paula et al. 2004). While unfed nymphal lone star ticks (*Amblyomma americanum*) may survive underwater for up to 19 weeks (Koch 1986), paralysis induced by hypoxia and depressed metabolic rate (Fielden et al. 2011) results in a loss of host-questing ability. Prolonged flooded rice paddies should therefore sustain very few, if any, actively questing ticks.

Similarly, chiggers are terrestrial (Söller et al. 2001). Likely due to the minute size of chiggers (~0.2 mm; Traub and Wisseman 1974), empirical studies on underwater survival of chiggers are limited, but support the hypothesis that rice paddies immersed in water for more than three months should be inhospitable for chiggers. For instance, *L. linhuaikonense*, *L. scutellare*, and *Walchia pacifica* can survive underwater for up to 60 days under the most favorable conditions, but most chiggers died within a month (Liu et al. 1997). Moreover, nymphal and adult trombiculid mites are free-living in the soil and feeding on the eggs and larvae of arthropods (Kawamura et al. 1995), so flooded habitat should hardly be a favorable environment for trombiculid mites. In Korea, scrub typhus occurred more commonly in women than in men, which was attributed in part to the fact that most men worked in rice paddies whereas women worked in dry fields (Kweon et al. 2009). Likewise, the much lower incidence rate of scrub typhus in western Taiwan likely reflects the much higher proportion of farmers working on rice

paddies than on dry fields (Kuo et al. 2011a). Both studies suggest a paucity of questing chiggers in flooded paddies. Although *Leptotrombidium* chiggers were reported occurring in rice fields in Japan and Thailand, these chiggers were in fact collected from areas in the vicinity of rice paddies that typically had abundant ground cover, including river banks, abandoned rice fields, orchards, or footpaths between fields (Tanskul and Linthicum 1999, Misumi et al. 2002, Takahashi et al. 2004), rather than from the flooded rice paddies per se. Habitat characteristics where these chiggers were recovered instead were more similar to fallow fields than to active rice fields.

Abandoned agricultural fields, filled with crop remnants and overgrown with secondary vegetation, provide good habitat for many rodent species. Favorable microhabitat and abundant rodent hosts in turn facilitate the proliferation of ticks (Sumilo et al. 2008) and chiggers (Traub and Wisseman 1974) in fallow fields. Therefore, abandonment of rice paddies in Taiwan, economically incentivized by globalization, appears to have inadvertently led to increased numbers of ticks and chiggers, with increased risk to humans from diseases such as spotted fever and scrub typhus likely occurring in and around fallow fields. It should be recognized, however, that the same land use transformation may suppress other vectors and associated diseases that proliferate in rice paddies (e.g., mosquitoes, malaria). The impact of land use change on human well-being is thus context dependent and contingent on the relative risks inflicted by different vectors. In Taiwan, mosquito-borne diseases, including malaria, lymphatic filariasis (LF), and Japanese encephalitis (JE), could be enhanced by rice cultivation, but malaria and LF have been eradicated since 1978 (Yeh et al. 2001), while a vaccination campaign has reduced the incidence of JE from high in the 1960s (approximately two cases per 100 000 people per year) to very low in recent years (0.03 cases per 100 000 in 1997; Wu et al. 1999). Therefore, abandonment of rice paddies is unlikely to further suppress malaria, LF, and JE, while it appears likely to increase incidence of spotted fever and scrub typhus. Temporal patterns in the incidence of spotted fever cannot be assessed because this disease is not reportable in Taiwan. However, scrub typhus has fluctuated annually from 1998 to 2007, with an overall positive trajectory (Centers for Disease Control, Taiwan 2008). Mean incidence of scrub typhus increased nearly 40% from 1998–2002 to 2003–2007 (from 1.26 to 1.76 cases per 100 000 people per year; population data: Department of Household Registration, Ministry of Interior 2008); whether this is related mainly to land use change or to climatic variability is not clear.

Periodic plowing is an agricultural policy intended to reduce farm pests in abandoned fields. Our data, however, revealed that rodents were not suppressed in plowed fields (Table 2). A further evaluation of the merits of periodic plowing on reducing farm pests is thus

required. Unexpectedly, periodic plowing can instead suppress ticks and chiggers, and likely decrease human vulnerability to spotted fever and scrub typhus. Other agricultural policies have similar unanticipated effects on human health. For instance, rice cultivation encouraged by a policy of price guarantee has led to a remarkable increase in mosquitoes in southern France shortly after World War II (Poncon et al. 2007). Our study supports research elsewhere that unveils the indirect and unintended consequences of agricultural activities on human health, and underscores the importance of interdisciplinary collaboration when drafting agricultural policies. For example, due to severe economic burdens, plowing subsidies may be postponed in Taiwan; this is likely to increase risks to tick- and chigger-borne diseases. This may be aggravated by a trend toward rural living, bringing more people into contact with agricultural lands; fields near houses usually are not cultivated but are plowed periodically. Abolishing plowing subsidies may expose these residents to elevated risks of vector-borne diseases.

Tick loads are a consequence of complex interaction among the environment, host density, and intrinsic characteristics of hosts, as exemplified by *I. scapularis* on *P. leucopus* and eastern chipmunks (*Tamias striatus*; Brunner and Ostfeld 2008). We observed dramatic seasonal variation of loads of *R. haemaphysaloides* on *A. agrarius*, with >20 times more ticks per host between August–September 2007 and October–November 2007 (Kuo et al. 2011b). We also found that plowing could have negative effects on tick loads, likely due to the reduced relative humidity in plowed soils. Nevertheless, after correcting for seasonal and plowing effects, tick loads were not affected by the four vegetative characteristics we have measured. This may reflect a lack of habitat specificity of *R. haemaphysaloides*, or it could reflect limited habitat variation in this study. In contrast to different habitat types among which tick abundance is known to differ (e.g., Lindsay et al. 1999, Estrada-Peña 2001, Guerra et al. 2002), we focused our study on abandoned agricultural fields, where the variation in habitat characteristics may not be large enough for a significant association to be identified. Further effort should integrate additional habitat types to more widely characterize the effects of habitat on tick abundance and the potential risks for tick-borne diseases.

We also failed to uncover any association between loads of larval or nymphal ticks on *A. agrarius* and trapping success of the three primary rodent hosts, *A. agrarius*, *R. losea*, and *B. indica*. Ticks spend >90% of life off hosts and their survival is determined largely by environmental stresses (Needham and Teel 1991). Consequently, hosts are essential for tick survival but are not deterministic in regulating tick abundance. Earlier studies have detected positive (Brunner and Ostfeld 2008), negative (Ostfeld et al. 1996, Krasnov et al. 2007), or no (Lindsay et al. 1999, Krasnov et al. 2007) association between tick loads and abundance of

rodent hosts. Such inconsistency can be accounted for by several mechanisms, which would vary with different tick–host associations (Krasnov et al. 2007). Likely the best means of disentangling the mechanisms decoupling tick loads and abundance of *A. agrarius* will be through experimental field studies. In any event, the current study demonstrates that rodent abundance is not necessarily associated with high tick infestation.

Somewhat surprisingly, determinants of chigger loads have not been well studied. Unlike most hard tick species that parasitize hosts during larval, nymphal, and adult stages, only larval trombiculid mites (i.e., chiggers) are parasitic. Consequently, relative to ticks, chigger loads should be less determined by host density than by other environmental factors. Reflecting this, it has been shown that chigger loads did not vary with host density (Kawamura et al. 1995). Neither did we find any association between chigger loads and trapping success of rodents. In contrast, the deterministic roles of temperature and rainfall are well understood (Kawamura et al. 1995), explaining why we observed a dramatic decline in chigger loads during the winter (Kuo et al. 2011b). However, while chigger loads may vary with habitats (Hubert and Baker 1963), we failed to detect any relation with vegetative characteristics. As noted above regarding habitat variation in tick loads, further study covering additional habitat types should help reveal the microhabitat requirement of chiggers.

In summary, our data clearly demonstrate that by accelerating transformations of land uses, economic globalization can influence risks to vector-borne diseases in unexpected ways. In Taiwan as well as in other countries, a combination of economic and agricultural policies, via greatly modifying ecological landscape, may have unanticipated impacts on public health, and underscore the importance of research on vector-borne diseases from broader perspectives.

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SUPPLEMENTAL MATERIAL

Appendix

Results on mean and 95% confidence interval of regression coefficients for environmental and host abundance variables in relation to tick and chigger loads on *Apodemus agrarius* (*Ecological Archives* A022-096-A1).