Infestation and seasonal fluctuation of chigger mites on the Southeast Asian house rat (Rattus brunneusculus) in southern Yunnan Province, China

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\textbf{A R T I C L E  I N F O}

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- Ascoschoengastia indica
- Leptotrombidium (L.) deliense
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- Leptotrombidium (L.) scutellare

\textbf{A B S T R A C T}

Chigger mites are the common ectoparasites of rodents and the exclusive vector of scrub typhus. The Southeast Asian house rat (Rattus brunneusculus) is an important reservoir host and infectious source of some zoonoses including scrub typhus. From April 2016 to March 2017, a 12-month consecutive investigation was made at Jingha village in southern Yunnan of China, which is an important focus of scrub typhus. The infestation and seasonal fluctuation of chigger mites on R. brunneusculus were studied based on the investigation. From 2,053 captured R. brunneusculus, a total of 99,221 chiggers were collected and identified as comprising 102 species with very high species diversity. The richness (S), diversity index (H'), evenness (E) and dominance index (D) of the chigger community on the rat varied in different months. Of the 102 chigger species, five main species accounted for 84.81% of the total chiggers (84,147/99,221). The five main chigger species were Walchia (W.) microptera (32.65%), Ascoschoengastia indica (24.68%), Leptotrombidium (L.) deliense (19.02%), W. (W.) turmalis (4.63%) and L. (L.) scutellare (3.83%). Of the five chigger species, L. (L.) deliense and L. (L.) scutellare are the most important vectors of scrub typhus in China. The five chigger species showed different patterns of seasonal fluctuation. The seasonal fluctuation of L. (L.) deliense belonged to summer-autumn type with the highest peak in July, but L. (L.) scutellare mainly appeared in winter and spring with the peak from January to February. The temperature and rainfall were two key factors which influenced the seasonal fluctuation of chigger mites.

1. Introduction

Chigger mites are a large group of arthropods with a unique mode of parasitism among medically-relevant arthropods and their larvae (often known as chiggers) are the exclusive ectoparasitic stage in their complex life cycle (Zhang et al., 2011; Walter et al., 2009; Santibáñez et al., 2015; Chaisiri et al., 2019). Most stages of chigger mites are edaphic creatures and some of them (deutonymphs and adults) are predators of some other arthropods (especially arthropod eggs) in the soil (Chaisiri et al., 2019; Shatrov and Kudryashova, 2006; Li et al., 1997). Chiggers are common ectoparasites on vertebrates (occasionally some invertebrates), and rodents and some other small mammals are their common hosts (Elliott et al., 2019; Daniel and Stelkonikov, 2009; Lv et al., 2019). As the exclusive vector of scrub typhus (tsutsugamushi disease) caused by the agent Orientia tsutsugamushi (Ot), some chigger species can transmit the disease among different hosts through their biting activity (Li et al., 1997; Santibáñez et al., 2015; Lv et al., 2019; Peng et al., 2018). In addition, some chiggers are suspected to be associated with the transmission of hemorrhagic fever with renal syndrome (HFRS) caused by different types of hantaviruses under Bunyaviridae (Wu et al., 1996; Li et al., 1997; Lv et al., 2019; Peng et al., 2018). Scrub typhus is a zoonotic disease potentially threatening human health and it is widely prevalent in Asian Pacific regions where more than one billion people are at risk of being infected and around one million new cases are reported annually (Bonell et al., 2017). In recent years, the prevalence of the disease in many places has shown a rapid increase, and the epidemic foci have been continuously expanding (Chaisiri et al., 2019; Elliott et al., 2019; Tilak and Kunte, 2019). Scrub typhus was previously believed to be only associated with Asian Pacific regions (Bonell et al., 2017), but it has probably spread to some other places of the world in...
recent years. For example, endemic scrub typhus has been reported from United Arab Emirates (Izzard et al., 2010) and Chile (Weitzel et al., 2016), and local transmission is suspected in Kenya (Masakhwe et al., 2018).

Scrub typhus is also widespread in China and its prevalence has been increasing with gradually expanded epidemic foci (Elliott et al., 2019; Wu et al., 2015). The disease is mainly prevalent in the vast areas south of the Yangtze River (e.g. Guangdong, Fujian, Hainan, Taiwan and Yunnan) (Su et al., 2012; Wu et al., 2013). Yunnan Province, especially southern Yunnan, is one of the main foci of scrub typhus in China (Yuan et al., 2018). There were 1208 cases of scrub typhus reported in Xishuangbanna prefecture in southern Yunnan between 2006 and 2017 (Yuan et al., 2018). The investigated site (Jingha village) of the present study is located in Xishuangbanna, an epidemic foci of scrub typhus.

The Southeast Asian house rat, Rattus brunneusculus, was named by Hodgson in 1845. Although some scholars considered R. brunneusculus a synonym of the Asian house rat, R. tanezumi Temminck, 1844 (Alfred, 2005; Ellerman, 1961; Wilson and Reeder, 2005), more scientists believe that R. brunneusculus is an independent rat species, which is obviously different from R. tanezumi in morphology (Dhananjoy et al., 2014a,b; Gao et al., 2017; Wang, 2003). The Southeast Asian house rat is not only an important agricultural and forestry pest, but also an important reservoir host and infection source of some zoonoses (plague, HFRS, and scrub typhus, etc.) (Chauhan and Saxena, 1987; Dong et al., 2014a,b; Gao et al., 2017; Wang, 2003). The capture of H. deliense isolated from some other “non-mite” impurities, the scurf and debris from the rats’ skin, under a stereo microscope, and then made into slide-mounted specimens with Hoyer’s medium. With the help of some relevant taxonomic literatures including taxonomic monographs and identification keys (Traub and Morrow, 1955; Traub and Evans, 1957; Nadchathram and Traub, 1971; Verscammen-Grandjean and Langston, 1976; Nadchathram et al., 1986; Goff et al., 1982; Res; 1990; Li et al., 1997; Fernandes and Kulkarni, 2003; Stekolnikov, 2013; Stekolnikov and González-Acuña, 2015; Chaisiri et al., 2016), the slide-mounted chiggers were identified to species under microscopes after dehydration and transparent process. The specimens of chiggers and representative rats were deposited in Institute of Pathogens and Vectors, Dali University, China.

2.3. Infestation statistics and analysis

On the basis of counting the total number of chigger species and the individuals of each chigger species, the constituent ratio (Cr), prevalence (Pv), mean abundance (MA) and mean intensity (MI) were used to calculate the infestations of the Southeast Asian house rat with chiggers. The Cr (%) is the percentage of each chigger species, Pv (%) the percentage of infested hosts (R. brunneusculus), MA the chiggers per examined rat host (mites/rat) and MI the chiggers per infested host (mites/rat) (Bush et al., 1997; Peng et al., 2018). Pearson’s linear correlation was used to analyze the relationship between infestations of R. brunneusculus with chiggers and climatic factors (temperature, humidity and rainfall) in 12 months (Lv et al., 2019).

2.4. Community structure analysis

The richness index (richness, S), Shannon-Wiener’s diversity index (H’), Pielou’s evenness (E) and Simpson’s dominance index (D) were used to describe the chigger community structure (Zhan et al., 2015).

\[ S = \sum S_i; \quad H' = -\sum_1^n \left( \frac{N_i}{N} \right) \ln \left( \frac{N_i}{N} \right); \quad E = \frac{H'}{\ln S}; \quad D = \sum_1^n \left( \frac{N_i}{N} \right)^2 \]

In the above formulas, \( S_i \) stands for chigger species i in the chigger community, \( N_i \) the number of chigger species i and \( N \) the total number of all chiggers.

2.5. Results

3.1. Infestation of the Southeast Asian house rat (Rattus brunneusculus) with chiggers

From 2,053 Southeast Asian house rats, a total of 99,221 chiggers were collected and they were identified as comprising 102 species with a high overall prevalence \( (P_M = 89.87\%) \), mean abundance \( (MA = 48.33\text{ mites/rat}) \) and mean intensity \( (MI = 53.78\text{ mites/rat}) \). The majority of chiggers were from May to October and the monthly fluctuation of all infestation parameters showed a slight peak in July \( (Cr = 12.32\%; P_M = 94.74\%; MA = 80.42\text{ mites/rat}; MI = 84.89\text{ mites/rat}) \) (Table 1, Fig. 1). The chigger community on R. brunneusculus also showed some monthly variations in species richness indices (S): 24–44, Shannon-Wiener's
species were for 84.81% (84,147/99,221) of the total chiggers. The five main chigger species were the most abundant and they accounted for 19.02% (18,867/99,221) of the total, 15.78% (13,733/99,221) of the total, and 10.14 mites/rat (7,565/747) of the total. The lowest chigger infestations occurred in December (Cr = 3.88%; Pm = 65.61%; MA = 6.65 mites/rat; MI = 10.14 mites/rat) (Table 4, Fig. 2).

Ascoschoengastia indica appeared throughout the year and it had an obvious seasonal fluctuation with two peaks. The first peak of Cr, MA

| Month | No. of hosts | Cr (%) | No. of mites | Cr (%) | Pm (%) | MA (mites/rat) | MI (mites/rat) |
|-------|--------------|--------|--------------|--------|---------|----------------|---------------|
| 1     | 182          | 8.87   | 6682         | 6.73   | 86.81   | 36.71          | 42.29         |
| 2     | 167          | 8.13   | 7131         | 7.19   | 87.43   | 42.70          | 48.84         |
| 3     | 182          | 8.87   | 8097         | 8.16   | 89.56   | 44.49          | 49.67         |
| 4     | 168          | 8.18   | 7119         | 7.17   | 86.31   | 42.38          | 49.10         |
| 5     | 184          | 8.96   | 11221        | 11.31  | 86.96   | 60.98          | 70.13         |
| 6     | 150          | 7.31   | 7725         | 7.79   | 94.67   | 51.50          | 54.40         |
| 7     | 152          | 7.40   | 12224        | 12.32  | 94.74   | 80.42          | 84.89         |
| 8     | 151          | 7.36   | 9003         | 9.07   | 91.39   | 59.62          | 65.24         |
| 9     | 141          | 6.87   | 7616         | 7.68   | 92.91   | 54.01          | 58.14         |
| 10    | 190          | 9.25   | 9400         | 9.47   | 91.05   | 49.47          | 54.34         |
| 11    | 197          | 9.60   | 6996         | 7.05   | 86.29   | 35.51          | 41.15         |
| 12    | 189          | 9.21   | 6007         | 6.05   | 92.59   | 31.78          | 34.33         |
| Total | 2053         | 100.00 | 99221        | 100.00 | 89.87   | 48.33          | 53.78         |

Table 1
Seasonal fluctuation of overall infestations of the Southeast Asian house rat (Rattus brunneusculus) with chiggers at Jingha village in southern Yunnan of China (2016–2017).

On the basis of calculating the constituent ratio (Cr), prevalence (Pm), mean abundance (MA) and mean intensity (MI), the seasonal fluctuations of infestations of R. brunneusculus with five main chigger species were summarized in Table 4.

Walchia (W.) micropelta could be found throughout the year with an irregular seasonal fluctuation. Its Cr, MA and MI were relatively high from March to July with a slight peak in May (Cr = 15.37%; MA = 27.06 mites/rat; MI = 36.61 mites/rat), and its Pm was highest in July (Pm = 84.21%). The lowest chigger infestations occurred in December (Cr = 3.88%; Pm = 65.61%; MA = 6.65 mites/rat; MI = 10.14 mites/rat) (Table 4, Fig. 2).

3.2. Seasonal fluctuation of five main chigger species on R. brunneusculus

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Ascoschoengastia indica appeared throughout the year and it had an obvious seasonal fluctuation with two peaks. The first peak of Cr, MA
Seasonal fluctuation of infestations of the Southeast Asian house rat (*R. bruneusculus*) with five main chigger species at Jingha village in southern Yunnan of China (2016–2017).

| Years | 2017 | 2016 | Total |
|-------|------|------|-------|
|       | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |
| Months |
| Examined hosts | 182 | 167 | 182 | 168 | 184 | 150 | 152 | 151 | 141 | 190 | 197 | 189 | 2053 |
| *W. (W.) microptera* mites | 2332 | 3337 | 4375 | 3101 | 4979 | 2314 | 3777 | 1408 | 1775 | 2239 | 1501 | 1257 | 32995 |
| Cr (%) | 7.20 | 10.30 | 13.51 | 9.57 | 15.37 | 7.14 | 11.66 | 4.35 | 5.48 | 6.91 | 4.63 | 3.88 | 100.00 |
| Pm (%) | 77.47 | 77.84 | 78.02 | 79.76 | 73.91 | 78.00 | 84.21 | 75.50 | 81.56 | 74.74 | 65.99 | 65.61 | 75.65 |
| MA | 12.81 | 19.98 | 24.04 | 18.46 | 27.06 | 15.43 | 24.85 | 9.32 | 12.59 | 11.78 | 7.62 | 6.65 | 15.78 |
| MI | 16.54 | 25.67 | 30.81 | 23.14 | 36.61 | 19.78 | 29.51 | 12.35 | 15.43 | 15.77 | 11.55 | 10.14 | 20.86 |
| *A. indica* mites | 1860 | 486 | 1069 | 1698 | 2494 | 1268 | 2457 | 4956 | 2861 | 3234 | 1639 | 378 | 24490 |
| Cr (%) | 7.59 | 1.98 | 4.37 | 6.93 | 10.18 | 5.18 | 10.03 | 20.24 | 11.68 | 13.57 | 6.69 | 1.54 | 100.00 |
| Pm (%) | 67.58 | 49.10 | 66.48 | 60.71 | 67.93 | 68.00 | 78.95 | 86.75 | 89.36 | 76.84 | 66.50 | 53.97 | 68.73 |
| MA | 10.22 | 2.91 | 5.87 | 10.11 | 13.55 | 8.45 | 16.16 | 32.82 | 20.29 | 17.49 | 8.32 | 2.00 | 11.93 |
| MI | 15.12 | 5.93 | 8.83 | 16.65 | 19.95 | 12.43 | 20.48 | 37.83 | 22.71 | 22.77 | 12.51 | 3.71 | 17.36 |
| *L. (L.) deliens* mites | 183 | 11 | 303 | 719 | 1186 | 3445 | 4553 | 927 | 1136 | 2605 | 2315 | 1484 | 18867 |
| Cr (%) | 0.97 | 0.06 | 1.61 | 3.81 | 6.29 | 18.26 | 24.13 | 4.91 | 6.02 | 13.81 | 12.31 | 12.27 | 7.87 |
| Pm (%) | 43.96 | 6.59 | 56.59 | 61.90 | 67.39 | 90.00 | 88.82 | 80.13 | 85.82 | 85.79 | 80.71 | 82.01 | 68.73 |
| MA | 1.01 | 0.07 | 1.66 | 4.28 | 6.45 | 22.97 | 29.95 | 6.14 | 8.06 | 13.71 | 11.75 | 7.85 | 9.19 |
| MI | 2.29 | 1.00 | 2.94 | 6.91 | 9.56 | 25.52 | 33.73 | 7.66 | 9.39 | 15.98 | 14.56 | 9.57 | 13.37 |
| *W. (W.) turmalis* mites | 118 | 165 | 293 | 626 | 1443 | 367 | 487 | 231 | 435 | 71 | 225 | 136 | 4597 |
| Cr (%) | 2.57 | 3.59 | 6.37 | 13.62 | 31.39 | 7.98 | 10.59 | 5.03 | 9.46 | 1.54 | 4.89 | 2.96 | 100.00 |
| Pm (%) | 33.52 | 41.32 | 53.85 | 57.74 | 63.04 | 51.33 | 65.13 | 50.33 | 73.05 | 23.16 | 39.59 | 33.33 | 47.78 |
| MA | 0.65 | 0.99 | 1.61 | 3.73 | 7.84 | 2.45 | 3.20 | 1.53 | 3.09 | 0.37 | 1.14 | 0.72 | 2.24 |
| MI | 1.93 | 2.39 | 2.99 | 6.45 | 12.44 | 4.77 | 4.92 | 3.04 | 4.22 | 1.61 | 2.88 | 2.16 | 4.69 |
| *L. (L.) scutellare* mites | 1060 | 1051 | 956 | 10 | 1 | 0 | 0 | 0 | 0 | 689 | 3798 |
| Cr (%) | 27.91 | 27.67 | 71.7 | 0.26 | 0.03 | 0.00 | 0.03 | 0.00 | 0.79 | 0.00 | 0.00 | 18.14 | 100.00 |
| Pm (%) | 66.48 | 61.68 | 67.58 | 2.38 | 0.54 | 0.00 | 0.66 | 0.00 | 16.31 | 0.00 | 0.00 | 59.26 | 23.77 |
| MA | 5.82 | 6.29 | 5.25 | 0.06 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 3.71 | 1.54 |
| MI | 8.76 | 10.20 | 7.77 | 2.50 | 1.00 | – | 1.00 | – | 1.30 | – | – | 6.15 | 7.78 |

and MI in May (Gr = 10.18%; MA = 13.55 mites/rat; MI = 19.95 mites/rat) was much lower than the second peak (highest peak of the whole year) in August (Gr = 20.24%; MA = 32.82 mites/rat; MI = 37.83 mites/rat). The highest PM, however, was in September (PM = 89.36%). The Cr, MA and MI decreased from September and reached the lowest level in December (Cr = 1.54%; MA = 2.00 mites/rat; MI = 3.71 mites/rat), but the PM was lowest in February (PM = 49.10%) (Table 4, Fig. 3).

*L. deliens* also appeared throughout the year and it had an obvious seasonal fluctuation with two peaks. The infestation parameters remained at a low level from January to April, and then rapidly rose from May on. The Cr, MA and MI reached the highest peak of the whole year (the first peak) in July (Cr = 24.13%; MA = 29.95 mites/rat; MI = 33.73 mites/rat). The highest PM, however, was in June (PM = 90.00%). The second peak of Cr, MA and MI appeared in October (Cr = 13.81%; MA = 13.71 mites/rat; MI = 15.98 mites/rat), but it was much lower than the first peak in July (Table 4, Fig. 4).
Walchia (W.) turmalis appeared throughout the year. Its Cr, MA and MI gradually increased from January to April, and then reached the highest peak of the whole year in May (Cr = 31.39%; MA = 7.84 mites/rat; MI = 12.44 mites/rat). The highest PM, however, was in September (PM = 73.05%). From June to December, most infestation parameters remained at a very low level, and they were the lowest in October (Cr = 1.54%; PM = 23.16%; MA = 0.37 mites/rat; MI = 1.61 mites/rat) (Table 4, Fig. 5).

All the infestation parameters of L. (L.) scutellare were very low from April to November, and they remained at the lowest level of the whole year. From December on, these parameters quickly increased, and then reached the peak in next January (Cr = 27.91%), February (MA = 6.29 mites/rat; MI = 10.20 mites/rat) and March (PM = 67.58%). From December to next March, all the infestation parameters maintained at a very high level (Cr: 18.14%–27.91%; PM: 59.26%–67.58%; MA: 3.65–6.29; MI = 6.15–10.20), forming an obvious seasonal fluctuation pattern (Table 4, Fig. 6).

3.3. Correlation between infestations of R. brunneusculus with chiggers and climatic factors

Pearson’s correlation analysis showed that the rainfall
(precipitation) was positively correlated with all the infestation parameters ($P_M$, $MA$ and $MI$) of $A$. indica ($r$: 0.776 for $P_M$, 0.815 for $MA$ and 0.812 for $MI$; $P < 0.01$), but negatively correlated with the $P_M$ of $L$. ($L$.) scutellare ($r = -0.596$, $P < 0.05$). The average temperature was positively correlated with all the infestation parameters ($P_M$, $MA$ and $MI$) of $W$. ($W$.) turmalis ($r$: 0.691 for $P_M$, 0.669 for $MA$ and 0.640 for $MI$; $P < 0.05$) and the mean intensity ($MI$) of $A$. indica ($r = 0.579$, $P < 0.05$), but negatively correlated with all the infestation parameters ($P_M$, $MA$ and $MI$) of $L$. ($L$.) scutellare ($r$: −0.734 for $P_M$, −0.725 for $MA$ and −0.804 for $MI$; $P < 0.05$). Although a negative correlation existed between the average humidity and the $Cr$ of $W$. ($W$.) micropelta ($r = -0.594$, $P < 0.05$), the humidity had little effect on the other 4 chigger species ($P > 0.05$) (Table 5).

4. Discussion

4.1. Species diversity and overall infestation of chiggers on the Southeast Asian house rat

There are more than 3,700 species of chigger mites widely distributed in the world and more than 400 species recorded in China (Li et al., 1997; Lv et al., 2019; Zhang et al., 2011). In the present study, a total of 102 chigger species were found on the Southeast Asian house rat ($R$. brunneusculus) with high overall infestations (Table 1). The 102
chigger species identified from such a single rat species at a localized area (Jingha village) are more than the chigger species recorded in some other provinces of China (e.g. 53 species in Fujian, 41 species in Hubei and 34 species in Sichuan) (Li et al., 2015; Wang and Liao, 1981; Yang and Liu, 2003), and even exceed all the chigger species in some countries (62 species in Nepal, 27 species in Afghanistan and 18 species in Poland) (Daniel et al., 2010; Daniel and Stekolnikov, 2009; Moniuszko and Makol, 2014; Peng et al., 2016). The result suggests that the Southeast Asian house rat has a great potential to harbor many chiggers with high species diversity. The overall infestation parameters of chiggers on Southeast Asian house rats showed a high level from May to October with a peak in July (Table 1, Fig. 1) and this is consistent with the fluctuation of scrub typhus in southern Yunnan (Yuan et al., 2018; Zhang, 2001). The abundant chiggers occurred in summer (July) may increase the risk of scrub typhus from the rats to human beings through the biting activity of chiggers.

4.2. Five main species of chigger mites on the Southeast Asian house rat

Of the 102 chigger species, five of them were the most abundant on *R. brunneusculus* and they are *Walchia (W.) micropelta*, *A. indica*, *L. (L.) deliense*, *W. (W.) turmalis* and *L. (L.) scutellare* (Table 3). Of the five main chigger species, *L. (L.) deliense* is the most powerful vector of scrub typhus and *L. (L.) scutellare* is the second major vector in China (Li et al., 1997; Lv et al., 2018; Su et al., 2012; Wu et al., 2013). Besides transmitting scrub typhus, *L. (L.) scutellare* is also suspected to potentially transmit hemorrhagic fever with renal syndrome (HFRS) (Li et al., 1997; Sanitóbas et al., 2015). *Leptotrombidium (L.) deliense* and *L. (L.) scutellare* often invade and sting humans and it is very easy for them to transmit the diseases from rats to humans (Li et al., 2005; Sanitóbas et al., 2015; Wu, 2005). *Ascoscoenogastia indica* is a potential vector of scrub typhus and it can carry *O. tsutsugamushi* (Chaisiri et al., 2019; Tilak and Kunte, 2019; Wu et al., 2013). The abundant *L. (L.) deliense*, *L. (L.) scutellare* and *A. indica* found on *R. brunneusculus* further increase the risk of scrub typhus from the rats to humans. To date there has been no evidence to show that *W. (W.) micropelta* and *W. (W.) turmalis* can be effective vectors of scrub typhus and some other zoonoses. The medical importance of abundant *W. (W.) micropelta* and *W. (W.) turmalis* found on *R. brunneusculus* remains unclear and further researches may be needed, including the isolation of the relevant pathogens from the mites.

4.3. Seasonal fluctuation of five main species of chigger mites

It is necessary to study the seasonal fluctuation pattern of chigger mites, which often influences the prevalence of scrub typhus (Candasamy et al., 2016; Li et al., 1997; Lv et al., 2019). In the present study, *Cr* and *MA* were selected as two effective parameters to depict the seasonal fluctuation curves of five main species of chiggers on the Southeast Asian house rat. The *Cr* is to reflect the percentage of each mite species in the mite community, and *MA* is to reflect the mites per examined rat (Peng et al., 2018). It is a good way to use these two parameters to illustrate the seasonal fluctuation patterns of chiggers and some other ectoparasites (Chen, 1980; Frances et al., 1999; Oorebeek and Klein-dorfer, 2008).

The five main chigger species had their own seasonal fluctuation patterns. Before the present study, some previous investigations had reported the seasonal fluctuations of *L. (L.) deliense*, *L. (L.) scutellare* and *A. indica* in some other provinces of China and some other countries (Frances et al., 1999; Kim et al., 2015; Li et al., 1997; Noda et al., 1996, 2013; Wu et al., 2013), but no literature was on the seasonal fluctuations of *W. (W.) micropelta* and *W. (W.) turmalis*. In the present study, the *Cr* and *MA* of *W. (W.) micropelta* and *W. (W.) turmalis* were relatively high from March to July with a slight peak in May and the mite had an irregular seasonal fluctuation without an obvious peak (Fig. 2). A negative linear correlation existed between average humidity and the *Cr* of *W. (W.) micropelta* (*P* < 0.05) and this suggests that the higher the humidity, the less the chigger mites. The result may imply that the high humidity may inhibit the survival and reproduction of *W. (W.) micropelta*. The result, however, is not consistent with the general opinions. According to the general opinions, the high humidity with much water vapor in the air is believed to be beneficial to the survival of most chigger mites (Clopton and Gold, 1993; Li et al., 1997). The seasonal fluctuation of *W. (W.) turmalis* belonged to spring type with a very prominent peak in May (Fig. 5). The average temperature was positively correlated with all the infestation parameters (*Pm*, *MA* and *MI*) of *W. (W.) turmalis* (*P* < 0.05). The positive correlation suggests that warm temperature may be beneficial to the survival, development and reproduction of *W. (W.) turmalis*, and this is in accordance with the situation of most chigger mites (Chaisiri et al., 2019; Clopton and Gold, 1993; Li et al., 1997; Sanitóbas et al., 2015).

Different from *W. (W.) micropelta*, *A. indica* showed an obvious
seasonal fluctuation though it could be found throughout the year. The seasonal fluctuation of A. indica seems to be spring-summer type. The mite show a small peak in May and the highest peak in August (Fig. 3). Some previous investigations from some other provinces of China showed that the seasonal peak of A. indica was in summer and autumn. The mite increased after May, decreased from August on and reached the lowest level (even no mites) in winter (Li et al., 1997). The seasonal fluctuation of A. indica in the present study is consistent with the previous records. In the present study, the rainfall (precipitation) was positively correlated with all the infestation parameters of A. indica (P < 0.01), and the average temperature was positively correlated with the MA of A. indica (P < 0.05). In the investigated site, it is hot and humid in summer with high temperature and rich rainfall (Lv et al., 2019; Sun et al., 2000). The positive correlations suggest that the high temperature with rich rainfall in summer may be beneficial to the survival and reproduction of A. indica.

As the most powerful vector of scrub typhus in China, Leptotrombidium (L.) deliense is believed to be the most major chigger species in the areas south of the Yangtze River (Lv et al., 2018; Su et al., 2012; Wu et al., 2013). In some regions of south Asian and southeast Asia, L. deliense is also a main vector of scrub typhus (Elliott et al., 2019; Santibáñez et al., 2015; Tilak and Kunte, 2019). Although the seasonal fluctuation curves of L. (L.) deliense vary in different geographical regions because of different latitude zones, altitudes and climates (Frances et al., 1999; Gentry et al., 1963; Lien et al., 1976), the mite usually has a preference to hot and humid weather (Candasamy et al., 2016; Frances et al., 1999; Li et al., 1997; Yuan et al., 2003; Zhang, 1994). In the laboratory, the warm temperature (18–28 °C) with high relative humidity (95–100%) is beneficial to the survival, development and reproduction of the mite (Li et al., 1997; Lv et al., 2018). In the present study, L. (L.) deliense was very abundant in summer, and the seasonal fluctuation of L. (L.) deliense belonged to summer-autumn type with the highest peak (the first peak) in July and the second small peak in October (Fig. 4). The result is similar to that in some other provinces of China and some other countries, in which the seasonal peak of L. (L.) deliense population often appeared in summer or/and autumn (Candasamy et al., 2016; Frances et al., 1999; Li et al., 1997; Yuan et al., 2003; Zhang, 1994).

Leptotrombidium (L.) scutellare is also a powerful vector of scrub typhus in China, which is second only to L. (L.) deliense (Li et al., 1997; Lv et al., 2018; Su et al., 2012). As a main chigger species in cold seasons, the seasonal fluctuation pattern of L. (L.) scutellare was opposite to that of L. (L.) deliense. The mite mainly appeared in winter and spring (from December to March) and its seasonal peak of Cr and MA was from January to February (Fig. 6). The result is consistent with that in some other provinces of China and some other countries (Choi et al., 2018; Li et al., 1997; Liu et al., 2004; Noda et al., 2013; Park and Shin, 2016; Pham et al., 1999; Wu et al., 2000; Yuan et al., 2003). Although the seasonal fluctuation curves of L. (L.) scutellare vary in different provinces of China, the basic pattern belongs to the autumn-winter type with the seasonal peak in cold seasons, late autumn and winter (Bang et al., 2008; Elliott et al., 2019; Santibáñez et al., 2015; Tilak and Kunte, 2019). The average temperature was negatively correlated with all the infestation parameters of L. (L.) scutellare (P < 0.05). The abundance of L. (L.) scutellare obviously increased with the decrease of temperature (Table 5). In addition, the rainfall (precipitation) was also negatively correlated with the P50 of L. (L.) scutellare (P < 0.05). The results suggest that the high temperature with rich rainfall is not good for the survival, development and reproduction of L. (L.) scutellare and the mite prefers a relatively cold and dry season to a hot and humid one.

5. Conclusions

The Southeast Asian house rat (R. brunneusculus) in southern Yunnan of China can harbor a variety of chigger species with high infestation. The five main chigger species on the rats are W. (W.) micropelta, A. indica, L. (L.) deliense, W. (W.) turmalis and L. (L.) scutellare, and they have different patterns of seasonal fluctuation. The seasonal fluctuation of the vector L. deliense belongs to summer-autumn type with the highest peak in July, and the vector L. scutellare mainly appears in winter and spring with the peak from January to February. Temperature and rainfall (precipitation) are two key factors which influence the seasonal fluctuation of chigger mites.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Alfred, J.R.B., 2005. State Fauna Series, Fauna of Manipur: zoological survey of India, Kolkata.
Bang, H.A., Lee, M.J., Lee, W.C., 2008. Comparative research on epidemiological aspects of tsutsugamushi disease (scrub typhus) between Korea and Japan. Jpn. J. Infect. Dis. 61, 148–150.
Bonell, A., Labella, Y., Newton, P.N., Crump, J.A., Paris, D.H., Foley, J., 2017. Estimating the burden of scrub typhus: a systematic review. PLoS Neglected Trop. Dis. 11, e05838.
Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: margolis et al. revisited. J. Parasit. 83, 575–583.
Candasamy, S., Ayyanar, E., Pailey, K., Karthikeyan, P.A., Sundararajan, A., Purushothaman, J., 2016. Abundance and distribution of trombiculid mites and Orientia tsutsugamushi, the vectors and pathogen of scrub typhus in rodents and shrews collected from Paducherry and Tamil Nadu, India. Indian J. Med. Res. 144, 940–945.
Chaisiri, K., Gill, A.C., Stekolnikov, A.A., Hinjoys, S., McGarry, J.W., Darby, A.C., Morand, S., Makepeace, B.L., 2019. Ecological and microbiological diversity of chigger mites, including vectors of scrub typhus, on small mammals across stratified habitats in Thailand. Anim. Microbiome. I https://doi.org/10.1186/s42523-019-0164-5.
Chaisiri, K., Stekolnikov, A.A., Makepeace, B.L., Serge, M., 2016. A revised checklist of chigger mites (Acari: Trombiculidae) from Thailand, with the description of three new species. J. Med. Entomol. 53, 321–342.
Chaubhan, N., Saxena, R., 1987. Annual reproductive cycle of the male field rat, Rattus rattus brunneusculus (Hodgson) in the hilly terrain of Mizoram. J. Bombay Nat. Hist. Soc. 84, 138–144.
Chen, X.P., 1980. Seasonal distribution of five chiggers in Tung-Shan district of Jiangsu province. Acta Entomol. Sin. 23, 99–101.
Choi, Y.J., Lee, I.Y., Song, H.J., Kim, J., Park, H.J., Song, D., Jang, W.J., 2018. Geographical distribution of Orientia tsutsugamushi strains in chiggers from three provinces in Korea. Microbiol. Immunol. 62, 547–552.
Clina, G., 2013. AVMA guidelines for the euthanasia of animal. J. Am. Vet. Med. Assoc. 242, 715–716.
Clifton, R.E., Gold, R.E., 1993. Distribution and seasonal and diurnal activity patterns of Euromarcia alfredii (Acari: Trombiculidae) in a forest edge ecosystem. J. Med. Entomol. 30, 47–53.
Daniel, M., Stekolnikov, A.A., 2009. Chigger mites (Acari: Trombiculidae) from Makalu provinces in Korea. Microbiol. Immunol. 53, 617–619.
Daniel, M., Stekolnikov, A.A., 2009. Chigger mites (Acari: Trombiculidae) from Makalu region in Nepal Himalaya, with a description of three new species. J. Med. Entomol. 46, 753–756.
Daniel, M., Stekolnikov, A.A., Hakimitarab, M., Saboo, A., 2010. Chigger mites (Acari, Trombiculidae) parasitizing small mammals in the Eastern Hindu Kush and some other Afghan areas. Parasitol. Res. 107, 1221–1233.
Dhananjay, C., Laisram, J.M., Singh, N.B., Leidang, T., Bjärrskogh, C., 2014a. Karyotype variation and species differentiation in the genus Rattus of Manipur, India. Afr. J. Biotechnol. 13, 4733–4744.
Dhananjay, C., Laisram, J.M., Singh, N.B., Wani, S.H., Bjärrskogh, C., Leidang, T., 2014b. Two new records of the genus Rattus from Manipur. Asian J. Anim. Sci. 9, 59–67.
Dong, W.G., Guo, X.G., Men, X.Y., Qian, T.J., Wu, D., 2009. Ectoparasites of Rattus stebni in areas surrounding Erhai lake in Yunnan province, China. Int. J. Parasit. Dis. 36, 19–25.
