

ARTHROPOD SPECIES DETECTION OF RICKETTSIAL INFECTION IN THE SOUTHERN OF VIETNAM, 2016

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Summary

We collected chigger mites at 7 survey sites in habitats of the national park, mangroves, nature reserves of the Southern on months the rainy season and dry season from 2015 - 2016.

Objectives: To determine chigger mites infection rate in rats and species composition, the distribution of chigger mites in rats, and to detect chigger mites of rickettsial infection which is parasitic on rats.

Results and conclusions: The common chigger mites infection rate in rats was 23.38% (143/600). In which *Suncus murinus* and *Mus musculus* were not infected chigger mites, the highest chigger mites infection rate in *Rattus rattus* was 36.36% (4/11) and *Rattus norvegicus* 32.87% (47/143). Collected 2,770 chigger mites individuals from 6 species, 3 genera, 1 family. Among them, *Ascoschoengastia* was a species of *Ascoschoengastia (L.) indica*; *Leptotrombicula* was 2 species of *Leptotrombidium (L.) deliense* and *Leptotrombidium (L.) striatum*; *Gahrlipeia* was 3 specie of *Gahrlipeia (W.) chinensis*, *Gahrlipeia (W.) lupella* and *Gahrlipeia (W.) parapacifica*. Total of 123 chigger mites samples (1,781 individual) were tested by PCR. Showed that the overall prevalence of *Orientia tsutsugamushi* was 1.62% (2/123). Where in *O. tsutsugamushi* in the specie of *Ascoschoengastia (L.) indica* was 4.67% (1/21) and the species of *L. (L.) deliense* was 1.85% (1/54). None of chigger mite samples positive with *Rickettsia* spp.

Key words: Chigger mites, rats, the South region in Vietnam.

INTRODUCTION

The larval stages (chiggers) of the genus *Leptotrombidium* are vectors of *Orientia tsutsugamushi*, causative agent of scrub typhus. This life - threatening disease is widely endemic in Asian Pacific regions where more than one billion people are at risk of acquiring the infection and around one million new cases are estimated to occur annually. In addition, although underreported and often misdiagnosed, trombiculiasis, defined as a dermatitis caused by the salivary secretion of biting chiggers, is present in America and Europe^[1].

The Southern of Vietnam is a region which

have biodiversity and ecosystems, such as Bu Gia Map National Park, Cattien National Park where are diverse and rich, organized immigration from many plants in the South East Asia. This is the place for the conservation of rare and precious species of fauna and flora and medicinal plants, and protection for headwater reservoirs of the hydropower reservoirs; U Minh Ha National Park, U Minh Thuong National Park are area of freshwater wetlands, comprising peat swamp forest, seasonally inundated grassland and open swamp. The soil layer is covered by a layer of peat, 1 to 3 m thick; The Cangio mangrove forest grew out of a comparatively recent brackish swamp with soil foundations created by the Sai Gon and Dong Nai Rivers. The development of the mangrove forest is dependent on high precipitation and a high density of rivers interweaving the area, which provide a rich and plentiful supply of alluvium in the estuarine regions. The soil formed in Cangio has been

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created by a combination of clay alluvial depositions, vitriolic processes and a brackish water table; Lung Ngoc Hoang Nature Reserve, Langsen Wetland Reserve are one of the typical models of wetland ecosystem, including mangrove forests, rice fields, seasonally waterlogged grasslands, etc.

Therefore, Our present study aims to capture information on (1) species composition and distribution of chigger mites parasitized on rats in the southern of Vietnam (2) and the presence of *Rickettsia* spp. and *Orientia tsutsugamushi* on chigger mites parasitized on rats in this region. Our preliminary data is a basis for the regional monitoring of chigger mites, in public prevention and control of chigger mites transmitted diseases.

SUBJECTS AND METHODS

Methods: Time study: From 2015 to 2016 (Collected chigger mites in rainy season 2015 and dry season 2016). Location of study: In the field of Cangio Mangrove Forest (Ho Chi Minh City), Langsen Wetland Reserve, Lung Ngoc Hoang Nature Reserve (Hau Giang province), Cattien National Park (Dong Nai province), U Minh Ha National Park (Ca Mau province), U Minh Thuong National Park (Kien Giang province).

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Subjects: Chigger mites (Trombicilinae). The hosts of chigger mites (Trombicilinae) which are rats at the survey sites. Rickettsiaceae bacteria.

Selection of sample and sample size

- The sample size for chigger mites species composition and distribution survey which were randomly conducted in the field, collecting all chigger mites samples on rats at the survey sites.

- The sample size for determining the rate of rickettsia infection in chigger mites which were made randomly on all chigger mites that collected from the field. Chigger mites individuals of the same species, parasitized on the same host or the same host group which were put on a sample, each sample has 10 - 20 chigger mites individuals.

Techniques used in research: Chigger mites were collected from rats, Examine carefully the thin skin areas such as the groin, leg, anus, ear, nose, eyelid, crest of rats for take chigger mites. Collected chigger mites were placed into plastic vials containing absolute ethanol and taken to the laboratory, where they were identified based on morphologic criteria following taxonomic keys (Nguyen Van Chau, 2007)^[2].

For the molecular detection and analysis of Rickettsia species, We pooled 10 - 20 chigger mites of the same species collected from one rat or rat species for DNA extraction, DNA was extracted by the ISOLATE II Genomic DNA Kit (Bioline, South Korea) according to the manufacturer's instructions. Multiplex PCR reactions were performed with total reaction volume was 50µl: 25µl 2x MyTaq HS Mix 2x (Bioline); 2.0µl Multiplex Primer 25nmol, to detect *Rickettsia* spp. and *Orientia tsutsugamushi*; 20µl distilled water; 3µl of mold DNA. PCR cycle: 94°C for 5 minutes, followed by 45 cycles: 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 45 seconds and ended at 72°C for 7 minutes (Applied Biosystem 2720 Thermocycle, America).

Table 1. Primers for groEL gene of Rickettsiaceae

No	Primer	Sequencing primers	Size	Rickettsiaceae gene segments: groEL
1	SF1	GATAGAAGAAAAGCAATGATG	229bp	<i>Rickettsia</i> spp.
	SR2	CAGCTATTTGAGATTTAATTTG		
2	TF1	ATATATCACAGTACTTTGCAAC	366bp	<i>Orientia tsutsugamushi</i>
	TR2	GTTCCCTAACTTAGATGTATCAT		

PCR products were detected by DNA electrophoresis with 2% agarose in TBE containing ethidium bromide for 30 minutes at 120 volts and bacteria are identified based on PCR product size on.



Research morality: The study was conducted on host species of chigger mites that did not cause any harms to humans. In addition, the study also discovered host species containing the vector - borne disease for humans and pathogens presenting in these chigger mites based on which recommendations could be made for local preventive centers.

Participants in the study who were trained on safety measures when conducting investigations, collection, treatment and identification. In cases, participant had infected pathogens (occupational diseases), They were entitled to benefits and regimes according to current regulations.

RESULTS

The individuals of rat were tested for which collected chigger mite at 7 sites in two surveys time by 2015 and 2016 that was 600 rat individuals of 8 species (Table 2). *Rattus norvegicus* was the highest individuals (143 individuals), following, *R. fulvescens* (113 individuals), *R. edwardsi* (98 individuals), *R. argentiventer* (90 individuals), *R. molliculus* (78 individuals), *Suncus murinus* (53 individuals), *Mus musculus* (14 individuals), *R. rattus* (11 individuals).

Table 2. Individuals of rats collected at study sites in the Southern region in 2015 - 2016

No	Rat species	Southern region (chigger mites infection rats/survey rats)							Totals
		Can Gio	Lang Sen	Lung Ngoc Hoang	Bu Gia Map	Cat Tien	U Minh Ha	U Minh Thuong	
1	<i>Suncus murinus</i>	0/10	0/2	0/13	0/5	0/10	0/3	0/10	0/53
2	<i>R. norvegicus</i>	4/17	11/14	6/26	4/29	6/18	9/26	7/13	47/143
3	<i>R. argentiventer</i>	0/0	9/25	7/24	0/0	0/1	4/15	4/25	24/90
4	<i>R. fulvescens</i>	3/14	7/12	2/14	2/9	0/17	12/34	7/13	33/113
5	<i>R. edwardsi</i>	1/10	2/2	1/12	2/18	5/14	12/28	4/14	27/98
6	<i>R. molliculus</i>	1/9	2/22	2/9	2/8	0/11	0/11	1/8	8/78
7	<i>Mus musculus</i>	0/2	0/2	0/5	0/2	0/0	0/1	0/2	0/14
8	<i>R. rattus</i>	0/0	0/0	0/0	2/5	1/5	0/0	1/1	4/11
Totals		9/62	31/79	18/103	12/76	12/76	37/118	24/86	143/600

The rats rate of common chigger mites infection was 23.38% (143/600). In which, *Suncus murinus* and *Mus musculus* were not infected chigger mites, the highest infection rate was *R. rattus* 36.36%, followed by *R. norvegicus* 32.87%, *R. fulvescens* 29.20%, *R. edwardsi* 27.55% %, *R. argentiventer* 26.67%, *R. molliculus* 10.26% (detailed results in tab. 2).

Chigger mites species composition parasitized on rats at survey sites in southern region 2015 2016

We have to collected 2,770 chigger mite individuals of 6 species, 3 genus, 1 family. In which, *Ascoschoengastia* was 1 species: *Ascoschoengastia (Laurentella) indica*; *Leptotrombidula* was 2 species: *Leptotrombidium (Leptotrombidium) deliense*, *Leptotrombidium (Leptotrombidium) striatum*; *Gahrlipeia* was 3 species: *Gahrlipeia (Walchia) chinensis*, *Gahrlipeia (Walchia) lupella*, *Gahrlipeia (Walchia) parapacifica*. The dominant species were *L. (L.) deliense*, *G. (W.) chinensis*, *As. (L.) indica* (Tab. 3).

Table 3. Species composition and individuals of chigger mites collected

No	Chigger mites species	Individuals
	Trombiculidae Ewing, 1944	
1	<i>Ascoschoengastia (Laurentella) indica</i> (Hirst, 1915)	448
2	<i>Gahrliepia (Walchia) chinensis</i> Chen et Hsu, 1955	678
3	<i>Gahrliepia (Walchia) lupella</i> Traub et Evans, 1957	299
4	<i>Gahrliepia (Walchia) parapacifica</i> Chen et Hsu, 1957	111
5	<i>Leptotrombidium (Leptotrombidium) deliense</i> (Walch, 1922)	1148
6	<i>Leptotrombidium (Leptotrombidium) striatum</i> Nadchatram et Traub, 1964	86
Totals		2.770

Distribution of chigger mites parasitized on rats at survey sites in southern region 2015 - 2016

Investigated 600 rats of 8 species at 7 sites of national parks, nature reserves, wetland reserve, mangrove forest in the Southern. The results show that, chigger mites parasitized on rats species in the following:

Table 4. Distribution of chigger mites parasitized on rats in the southern

No	Chigger mites species	Rats species (Host)
1	<i>As. (L.) indica</i>	<i>Rattus fulvescens</i> , <i>R. edwardsi</i> , <i>R. argentiventer</i> , <i>R. norvegicus</i> , <i>R. rattus</i> .
4	<i>G. (W.) chinensis</i>	<i>R. edwardsi</i> , <i>R. fulvescens</i> , <i>R. molliculus</i> .
5	<i>G. (W.) lupella</i>	<i>R. argentiventer</i> , <i>R. norvegicus</i>
6	<i>G. (W.) parapacifica</i>	<i>R. molliculus</i>
7	<i>L. (L.) deliense</i>	<i>R. edwardsi</i> , <i>R. argentiventer</i> , <i>R. norvegicus</i> , <i>R. molliculus</i> , <i>R. fulvescens</i> , <i>Rattus rattus</i>
8	<i>L. (L.) striatum</i>	(<i>R. fulvescens</i> , <i>R. edwardsi</i> , <i>Rattus rattus</i>)

R. norvegicus, *R. fulvescens*, *R. edwardsi* and *R. rattus* were the main hosts of many species of chigger mites; The remaining rats were the hosts of some chigger mites species (Tab. 4).

During the two survey times in rainy season (2015) and the dry season (2016) at 7 survey sites in the Southern, the distribution of chigger mites parasitized on rat as shown in Figure. 1:

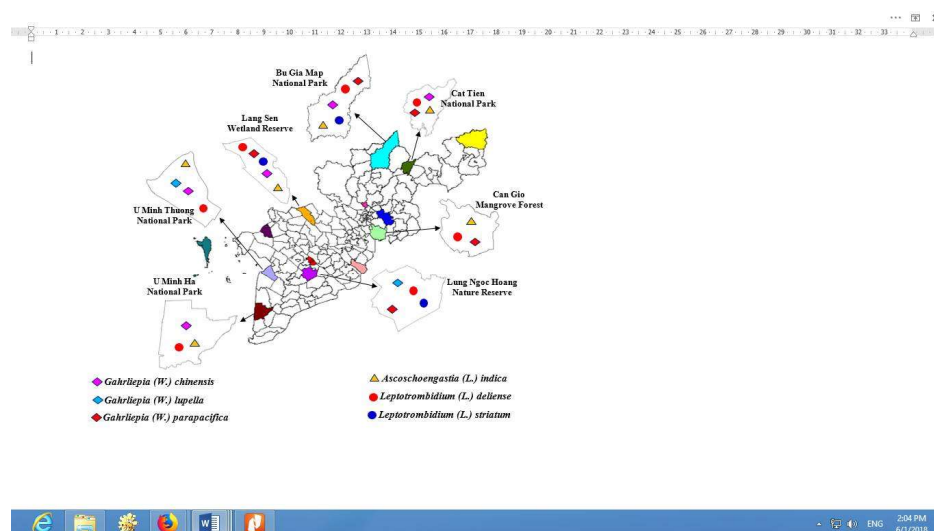


Figure. 1. Distribution diagram of chigger mites at 7 survey sites in 2015 - 2016



Chigger mites species distributed widely in region which were *L. (L.) deliense* at 7/7 survey sites, *As. Indica* distributed at 6/7 sites, *G. (W.) chinensis* and *G. (W.) parapacifica* distributed at 5/7 sites. The remaining chigger mite species were narrow distribution: *G. (W.) lupella* was only distributed at 2/7 survey sites and *L. (L.) striatum* was distributed at 3/7 sites (Figure. 1).

Pathogen present on chigger mites parasitized on rats at survey sites in southern 2015 - 2016

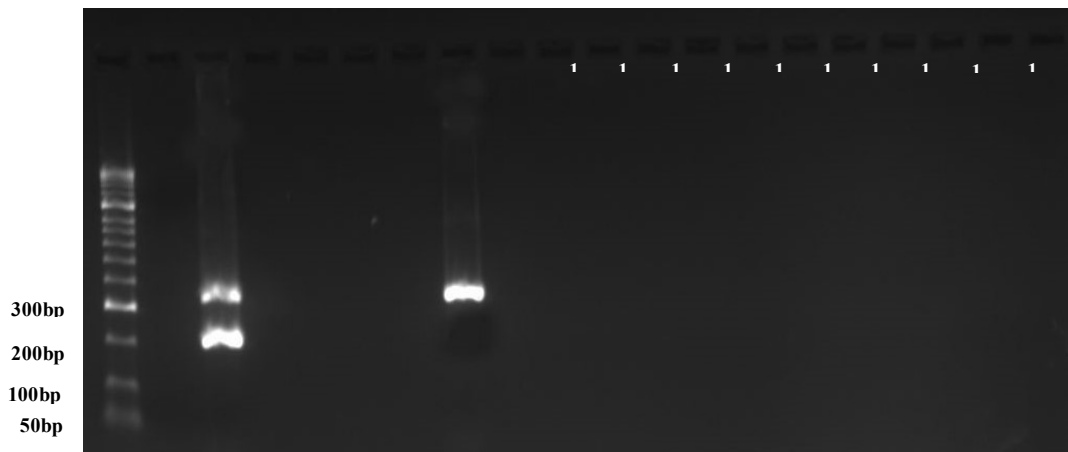


Figure 2. The results of the PCR detected DNA of *O. tsutsugamushi*

Note: Well 1: DNA bands (50 bp); well 2: negative; well 3: positive of *Rickettsia* spp. (299 bp) and *O. tsutsugamushi* (366 bp); Wells 4 - 20: chigger mites samples; Well 8: chigger mite sample was positive with DNA of *O. tsutsugamushi* (299 bp).

Table 5. Analysis results of rickettsiaceae on chigger mites by PCR technique

NO	Species of chigger mites	Samples (individuals)	Sample and rate (%) (+) with <i>Rickettsia</i> spp.	Sample and rate (%) (+) with <i>O. tsutsugamushi</i>
1	<i>As. (L.) indica</i>	21 (297)	0	1 (4,76)
2	<i>G. (W.) chinensis</i>	23 (384)	0	0
3	<i>G. (W.) lupella</i>	13 (219)	0	0
4	<i>G. (W.) parapacifica</i>	6 (84)	0	0
5	<i>L. (L.) deliense</i>	54 (736)	0	1(1,85)
6	<i>L. (L.) striatum</i>	6 (61)	0	0,00
Totals		123 (1.781)	0	2 (1,63)

Analysis of 123 chigger mites samples (each sample contains 10 - 20 individuals) with 1,781 chigger mite individuals to determine the pathogen which was *Rickettsia* spp. and *Orientia tsutsugamushi*. The results in Table 5, Fig. 2 show that: Chigger mites collected on rats in the Southern which was infection rate of *Orientia tsutsugamushi* 1.62%. Of which, 1/21 sample of *Ascoschoengastia (L.) indica* infected *O. tsutsugamushi* with infection rate 4,76% and 1/54 sample of *L. (L.) deliense* infected *O. tsutsugamushi* with infection rate 1,85%. There wasn't positive sample for *Rickettsia* spp.

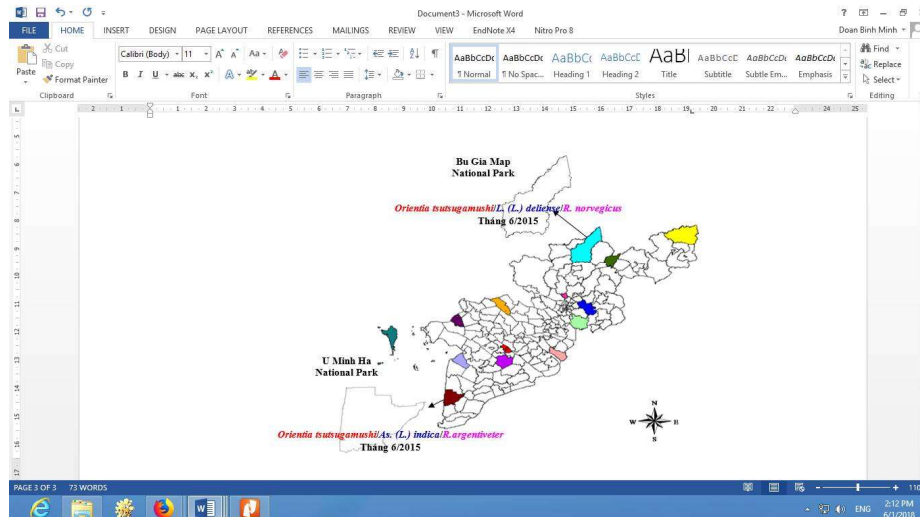


Figure 3. Distribution diagram of chigger mites parasitized on rats at 7 survey sites in southern of Vietnam (2015 - 2016)

At 7 survey sites, two positive chigger mite samples with DNA of *O. tsutsugamushi* which were *As. (L.) indica* (parasitized on *R. argentiveter*) and *L. (L.) deliense* (parasitized on *R. norvegicus*). They were found at two survey site: Bu Gia Map National Park, Binh Phuoc province in June 2015 and U Minh Ha National Park, Ca Mau province in June 2015 (Figure 3).

DISCUSSIONS

We have collected 600 rat individuals and 2,770 chigger mite individuals. Among them, the rate of *R. rattus* infected chigger mite was 36.36%, *R. norvegicus* 32.87%, *R. fulvescens* 29.20%, *R. edwardsi* 27.55%, *R. argentiveter* 26.67%, *R. molliculus* 10.26%, *Suncus murinus* and *Mus musculus* weren't infected. From the results of the above analysis, we have determined the species composition, distribution of chigger mites parasitized on rats as well as the infection status of rickettsiaceae on chigger mites in the Southern region of Vietnam as follows:

The chigger mites species composition parasitized on rat in the Southern region 2015 - 2016

Chigger mite species composition in the Southern region (2015 - 2016) which was lower than chigger mites species composition in other regions such as province of Quangbinh and Ha Tinh (Nguyen Van Chau, 2005)^[3], collected 16 species. Con Dao Island (Vu Duc Chinh, 2006)^[4], collected 7 species. Province Quang Nam and Kon Tum (Nguyen Van Chau, 2004)^[5], collected 6 species. However, chigger mite composition species in June 2015 and U Minh Ha National Park, Ca Mau province in June 2015 (Figure. 3).

In the Southern was collected only in rats while other studies were collected chigger mites on other host groups (wild birds, raising birds, Garbage and animal nest...).

Distribution of chigger mites parasitized on rats at survey sites in southern 2015 - 2016

Investigated 600 rats of 8 species at 7 sites of national parks, nature reserves, mangrove forest in

the Southern. We collected 2,770 chigger mites individuals. Of which, *R. norvegicus*, *R. fulvescens*, *R. edwardsi* and *R. rattus* were the main hosts of many species of chigger mites. Chigger mites weren't parasitized on *Suncus murinus* and *Mus musculus*. Chigger mites species distributed widely in region which were *L. (L.) deliense*, *As. Indica*, *G. (W.) chinensis* and *G. (W.) parapacifica*. The remaining chigger mite species were narrow distribution which were *G. (W.)*



lupell, *L. (L.) striatum*. Langsen wetland reserve and Bu Gia Map National Parks had the highest chigger mites species composition (5 species). Following, Cat Tien National Parks (4 species), U Minh Thuong National Parks (4 species), Lung Ngoc Hoan nature reserves (4 species); Con Dao island (3 species), Langbiang (3 species) and Can Gio mangrove forest (2 species), U Minh Ha National Parks (2 species).

According to Nguyen Van Chau (2007)^[2], the Central South - Southern Region had 62 species (58.5% of total chigger mite species of Vietnam), 17 genus, 2 subfamily; where was the region with the highest number of genus and species. Of them, *Leptotrombidium* and *Gahrlipeia* genus had dominance of the species number.

Pathogen present on chigger mites parasitized on rats in southern region 2015 - 2016

A total of 123 chigger mites samples (1,770 individuals) were determined of *Rickettsia* spp. and *O. tsutsugamushi* by PCR. Two samples were positive for DNA of *O. tsutsugamushi* and there wasn't samples which was positive with DNA of *Rickettsia* spp. (Tab. 5 and Fig 3). The rate of chigger mites infected *O. tsutsugamushi* which was collected on rats in the Southern region, it was 1.67%. Two chigger mites species infected DNA of *O. tsutsugamushi* they were *L. (L.) deliense* and *As. (L.) indica*. According to Nguyen Van Chau (2007)^[2] among the chigger mites species discovered in Vietnam, there are 5 species that are vector - borne - disease: *L. (L.) deliense*, *L. (L.) akamushi*, *L. (L.) scutellare*, *As (L.) audyi*, and *As. (L.) indica*. In addition, two species of *G. (G.) pintanensis* and *G. (G.) parapacifica* isolated *Rickettsia tsutsugamushi* (*O. tsutsugamushi*). The rate of chigger mites infected *O. tsutsugamushi* in this study was lower than other studies such as Hee Il Lee et al., (2013). The rate of chigger mites infected *Orientia tsutsugamushi* which was 2.7%

at 9 survey sites in Korea^[6]. Meng Zhang (2013). Detected 29/164 chigger mites which were positive with *O. tsutsugamushi* in Shandong province, China^[7].

CONCLUSIONS

The rate of rats infected chigger mites at survey sites in southern of Vietnam which was 23.38% (143/600). Of which, the rate of *R. rattus* infected chigger mites which was 36.36%, *Rattus norvegicus* 32.87%, *R. fulvescens* 29.20%, *R. edwardsi* 27.55%, *R. argentiventer* 26.67%, *R. molliculus* 10.26 %; *Suncus murinus*, *Mus musculus* weren't infected chigger mites.

The species composition of chigger mites (*Trombiculidae*) parasitized on rats in the Southern (collection 2015 - 2016), consists of 3 genus, 6 species: *Ascoschoengastia (Laurentella) indica*, *Gahrlipeia (Walchia) chinensis*, *G. (W) lupella*, *G. (W.) parapacifica*, *Leptotrombidium (Leptotrombidium) deliense*, *L. (L.) striatum*. The dominant species were *L. (L.) deliense*, *As. (L.) indica*, *G. (W.) chinensis*.

Distribution of chigger mites parasitized on rats in the Southern region: chigger mites were mainly found on *Rattus norvegicus*, *R. fulvescens*, *R. edwardsi*, *R. rattus*; The distribution of chigger mites parasitized on rats at the survey sites which was very different. There were 3 species of *L. (L.) deliense*, *G. (W.) chinensis* and *As. (L.) indica* which were widely distributed from 5 to 6 survey sites, the remaining species distributed only from 2 to 3 survey sites.

The rate of chigger mites parasitized on rats infected DNA of *O. tsutsugamushi* which was 1.63% (2/123) and the percentage of *L. (L.) deliense* infected DNA of *O. tsutsugamushi* which was 1, 85% (1/54), and *As. (L.) indica* infected DNA of *O. tsutsugamushi* which was 4.76% (1/21).

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