

Original Article

Ectoparasites Diversity on Rodents and Shrews at Scrub Typhus Endemic Vellore District of Tamil Nadu, India

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Abstract

Background: Rodents and shrew living in the vicinity of human beings perform significant role to harbor different species of ectoparasites and thus act as the reservoir host for the spread of diseases to human and animals.

Methods: This study was undertaken to determine the species composition of the medically important ectoparasites present in the scrub typhus affected places of Vellore District using wonder and Sherman traps for trapping of live Rodent/Shrew during September 2017 to August 2018.

Results: Rodent/shrew hosts belonged to two Families, three sub families and five genera and five different species. These animals carried 23 species of ectoparasites including 17 trombiculid mites, two non-trombiculid mites, two ticks and two fleas. A total of 940 chigger mites were collected which belonged to three Tribes, six Genera, two Subgenera and 17 species. Adult mites collected belonged to two families, three genera and three species. Ticks collected were classified under one family, two genera and two species. Fleas fall under one family, one subfamily, one tribe, one genus and two species.

Conclusion: This is the first record of 14 species of chigger mites and three species of adult mites from Vellore District, Tamil Nadu. Surveillance of these keystone ectoparasites helped to identify the medically important disease vectors causing acari-borne zoonotic diseases.

Keywords: Diversity; Ectoparasites; Rodents; Scrub typhus; Tamil Nadu-India

Introduction

Mites, ticks, lice, and fleas are important haematophagous arthropod ectoparasites found associated with rodents and play vital role in the transmission of different vector-borne diseases like scrub typhus, plague etc (1–3). Rodents and shrews that live nearby human dwellings are considered as the synanthrope species causing major role in the transmission of diseases to human (4). In the rodents and shrews, chigger mites are predominant organism which are the larval stages of mites belonging to Trombiculidae family comes under subclass Acari of class Arachnida, transmitting Scrub typhus disease. Many acarines act as the vectors of zo-

onotic diseases and live in close association with different animal host. In India, two major traps are commonly used, which are Sherman and wonder traps to capture rodents and shrews alive to harvest different ectoparasites (5).

Scrub typhus was a major out-break disease during 2017 in Tamil Nadu. A study on serological evidence of distribution of spotted fevers, scrub typhus and other typhus fevers transmitted through ectoparasites of rodents were already reported in Tamil Nadu (6). An account of the abundance of ectoparasites on the host at Tamil Nadu and Pondicherry in different seasons was documented already (7).

Based on our earlier study, the prevalence of chigger mites was reported in the scrub typhus affected areas of Trivandrum District, Kerala State, India (8).

Even though maximum number of scrub typhus cases was reported from Vellore District, Tamil Nadu, very little study has been undertaken to understand the diversity of the ectoparasites from the different rodents/shrew present in Vellore District. A comprehensive survey on the ectoparasites was undertaken in the scrub typhus endemic Vellore District of Tamil Nadu to identify the different species of ectoparasites present here and to bring out the medically important ectoparasites from this area. This study brings out for the first time the presence of a variety of diverse ecto-parasites of zoonotic importance available in Vellore District, Tamil Nadu.

Materials and Methods

Site selection and Trapping

Vellore district situated in Tamil Nadu state of India is one of the 37 districts in the Tamil Nadu state. It is one of the 11 districts that form the North region of Tamil Nadu. Vellore District lies between 12° 15' to 13° 15' North latitudes and 78° 20' to 79° 50' East longitudes in Tamil Nadu State. The geographical area of this district is 5920.18Km² and a housing population of 3, 936,331 as reported by the 2011–2022 census (9). The average annual temperature in Vellore is 27.9 °C and the annual rain fall is 971mm.

Based on the earlier history of scrub typhus, as the maximum numbers of scrub typhus cases (80–161 from 2013 to 2016-Source DPH and PM Chennai, Tamil Nadu) were reported in Vellore District it was selected for this study. In Vellore District, in consultation with the local health officials seven localities were selected such as Ponnai Poigai, Annaicut, Munjurpet and Lalapet (rural areas) and Nowlok Thoppu and Vellore town (urban areas) for the collection of ecto-parasites.

Sample collection

The ectoparasites collection was made from, trapping of rodents and shrew was conducted based on scrub typhus affected areas during 2018 (Fig. 1). Sherman traps and Wonder traps were placed in the study villages to capture rodents and shrew, found freely close to human habitats. Traps were set in the evening (6.00 pm) and the traps were retrieved the next day morning (7.00am). Rodents were anesthetized (7) for the collection of various ectoparasites. The ectoparasites were harvested from the captured rodents/shrews (8).

Identification

All the ectoparasites collected from these animals were preserved in 80% ethanol, transferred to clearing agent, mounted in Hoyer's medium and examined under the microscope for species level identification using standard taxonomical keys (10–15). All collected ectoparasites specimens were preserved in the slide and deposited in Mosquito Museum Entomology Laboratory of ICMR-Vector Control Research Centre Field Station, Madurai, Tamil Nadu.

Ethics

This study was approved by the Institutional Animal Ethics Committee (IAEC) of Madurai Medical College Madurai, Tamil Nadu, India (Ref. No. 2339/E1/5/2017 dated 23. 3.2017; S.No. 10).

Data analysis

The data analysis was performed using SPSS Ver.15 (Statistics Package for Social Sciences).

Results

This study showed distribution pattern of chiggers and mites in Vellore District, Tamil Nadu. A total of 350 traps were placed, keeping equally 50 numbers of traps at each rodent trapping site. There were only 36 traps found to be positive capturing 56 rodents/ shrew be-

longing to five species *Rattus rattus*, *Suncus murinus*, *Tatera indica*, *Bandicota bengalensis* and *Mus musculus*. The results of the collected rodent/shrew hosts were identified as comprising of two families (Muridae, Soricidae), three subfamilies (*Gerbillinae*, Murinae and *Soricinae*) and five genera (*Tatera*, *Mus*, *Bandicota*, *Rattus* and *Suncus*). Rodent trapped per trap was 1.56.

These five different species of rodents/shrew collected from Vellore District of Tamil Nadu carried 23 species of ectoparasites included 17 trombiculid mites, two non-trombiculid mites, two ticks and two fleas representing five families namely Trombiculidae (17), Laelapidae (1), Dermanyssidae (1), Ixodidae (2), and Pulicidae (2). Large sampling size helped to gather many species of chigger mites. A total of 940 chigger mites (94.76%) were harvested predominantly from rats/shrew followed by 45 fleas (4.5%), 4 ticks (0.4%) and 3 adult mites (0.3 %). Chigger index, flea index, tick index and mite index were 16.79, 0.80, 0.07 and 0.05 respectively (Table 1). The collected 940 chigger mites from all the rodent/shrew hosts were identified belonging to one family (Trombiculidae), subfamily Trombiculinae, which belonged to three tribes (Trombiculini, Schoengastiini and Gahrlipeini), six genera (*Leptotrombidium*, *Trombicula*, *Neotrombicula*, *Schoengastia*, *Ascoschoengastia* and *Schoengastiella*), two subgenera (*Leptotrombidium* and *Ericotrombidium*) and 17 species. Only three adult mites were collected belonging to two families (Laelapidae and Dermanyssidae) two genera (*Echinolaelaps* and *Dermanyssus*) and two species. Similarly, only four ticks were collected in this study classified under one family (Ixodidae) two genera (*Haemophysalis* and *Liponyssoides*) and two species. This study showed a total of 45 fleas coming under one family, one subfamily (Pulicidae), one tribe (Xenopsyllini), one genus (*Xenopsylla*) and two species. Species of ectoparasites recovered from field rodents and shrew at Vellore District, Tamil Nadu is given in Table 2.

Maximum 273 chiggers were collected from single rodents *Rattus rattus*. The dominant numbers of chigger per rodent was recovered from the village Munjurpet (141) and Anaikattu (140). In the urban sites, from the area Nowlok Thoppu, 464 chiggers were collected. No flea was collected at the urban site. Maximum number of fleas per rodent (11) was collected from Munjurpet village. Adult mites and ticks were recovered at a low number from both urban and rural sites. A taxonomic checklist of available ecto-parasites and rodents/shrew are listed in the Appendix: 1 and 2.

Ectoparasite infestation rate on the rodent *Rattus rattus* was 65%, followed by *Suncus murinus* (60%) and *Mus musculus* 33%. Only one *Bandicota bengalensis* was captured and infested with only 18 chiggers. Number of ectoparasites collected in a shrew *Suncus murinus* was 58.8, followed by 23.2 in *Rattus rattus*, 18 in *Bandicota bengalensis* and 15 in *Mus musculus*. Infestation rate for chiggers in rural area was 87.47% and in the urban locality was 99.23%. Fleas reported only from rural areas showed infestation rate 9.89%. Ticks were collected both in rural (Infestation rate 0.22%) and urban areas (0.46%). Adult mites were prevalent in both rural (0.22%) and in urban areas (0.31%). Fleas showed a significant difference between its distribution pattern in rural (45) and urban areas (no fleas at urban) ($t = -1.927$, $df = 348$, $p < 0.05$). However, there was no significant difference between the presence of chiggers on rodents between rural and urban sites ($t = -0.173$, $df = 348$, $p > 0.05$), for ticks ($t = -0.183$, $df = 348$, $p > 0.05$) and for adult mites ($t = -1.467$, $df = 348$, $p > 0.05$) (Table 1).

From the total collection, 67% mites, 25% fleas, 5% ticks and 3% other mites were collected at rural collection sites. Likewise, 82% mites, 9% ticks and 9% adult mites were collected at urban trapping sites. More rodents were trapped at rural villages mainly from Munjurpet (15) and Anaikattu (14) villages. Ectoparasites collection was dominant in an Urban site

Nowlok Thoppu (47%). Among the hosts *Rattus rattus* was trapped dominant (71%). Chiggers were recovered more at Vellore study sites (94.76%).

The chigger species *Leptotrombidium deliense* and *Leptotrombidium indicum* were found in all species of rodents and shrews except *Bandicota bengalensis* and *Mus musculus*. Next to the chiggers *Leptotrombidium deliense* and *Leptotrombidium indicum*, *Schoengatiella ligula* were collected more in *Rattus rattus*, *Bandicota bengalensis* and *Tatera indica*. The fleas *Xenopsylla cheopis* was found only in *Rattus rattus* and *Mus musculus* at rural areas. The fleas *Xenopsylla astia* was found only in *Rattus rattus*, *Bandicota bengalensis* and *Tatera indica*. There was no significant difference between rural and urban localities in rodent positivity ($t = -0.406$, $df = -54$, $p > 0.05$). This is the first record of 14 different species of chiggers and 3 species of adult mites from Vellore District, Tamil Nadu (Table 2).

Table 1. Areawise ectoparasites collected from rodents and shrew at Vellore District, Tamil Nadu, India

Area	Rural	Urban	Total
No of traps fixed	205	100	350
No of traps positive	27	9	36
Trap positivity rate	10.8	9.0	10.3
No. of Rodents/ Shrew collected	45	11	56
No. of rats positive for Chiggers	24	9	36
Percentage positive for Chiggers	53.3	81.8	64.3
No. Chiggers collected	398	542	940
Chigger Index	8.8	58.3	16.79
No. of rats positive for fleas	9	0	9
Percentage positive for fleas	20.0	0.0	64.3
No. Fleas collected	45	0	45
Flea Index	1.0	0.0	0.80
No. of rats positive for ticks	2	1	3
Percentage positive for ticks	4.4	9.1	64.3
No. Ticks collected	3	1	4
Tick Index	0.04	0.27	0.09
No. of rats positive for mites	1	1	2
Percentage positive for Adult mites	2.2	9.1	3.6
No. adult mites collected	1	2	3
Adult Mite Index	0.02	0.1	0.05

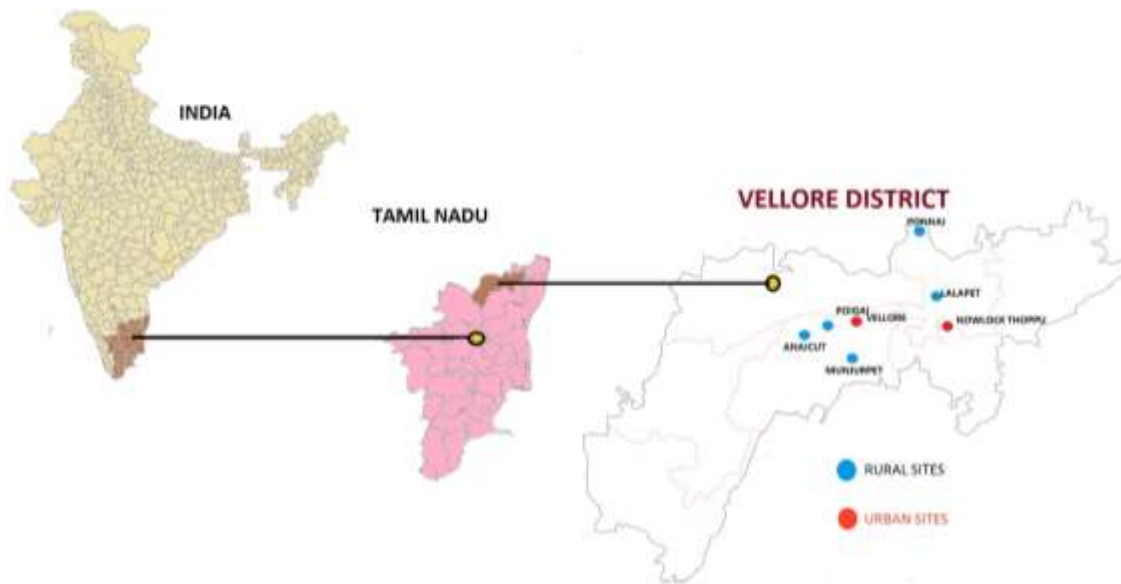


Fig. 1. Study areas selected in Vellore District, Tamil Nadu, India

Table 2. Species wise ectoparasites recovered from field rodents and shrew trapped at Vellore District, Tamil Nadu, India

Location	Rural							Urban							Ectoparasites species recorded in present survey							
	Villages		A1		A2		A3			A4			A5		A6		A7		R	R	R	#
Ectoparasites/Hosts	Sm	Rr	Sm	Rr	Rr	Ti	Bb	Mm	Sm	Rr	Rr	Rr	Sm	Rr	Rr	Sm	Rr	(7)	(14)	(13)	#	##
Chigger mites																						
<i>Leptotrombidium deliense</i>	•	•				•		•	•	•		•	•	•	•			•			•	
<i>Leptotrombidium rajasthanense</i>	•	•							•	•											•	FR
<i>Leptotrombidium keukenschrijveri</i>			•																		•	FR
<i>Leptotrombidium indicum</i>			•	•	•	•			•	•	•	•	•	•							•	FR
<i>Leptotrombidium delimushi</i>																					•	FR
<i>Leptotrombidium insigne</i>																					•	
<i>Leptotrombidium kulkarni</i>																					•	FR
<i>Leptotrombidium spilleti</i>																					•	FR
<i>Leptotrombidium dehradunense</i>									•												•	FR
<i>Leptotrombidium jayewickremei</i>									•												•	FR
<i>Schoengastia kanhaensis</i>																					•	FR
<i>Schoengastiella argalea</i>									•												•	FR
<i>Schoengastiella ligula</i>						•	•	•													•	FR
<i>Schoengastiella.sp</i>		•																			•	
<i>Schoengastia sp.</i>																					•	
<i>Ascoschoengastia indica</i>																					•	FR
<i>Trombicula hypodermata</i>									•	•											•	FR
<i>Neotrombicula.sp</i>		•																			•	FR
Adult Mites																						
<i>Echinolaelaps echininus</i>																					•	FR
<i>Dermanyssus gallinae</i>																					•	FR
<i>Liponyssoides sanguineus</i>																					•	FR
Ticks																						
<i>Haemophysalis sp</i>									•			•									•	
<i>Rhipicephalus turanicus</i>																					•	
Fleas																						
<i>Xenopsylla cheopis</i>		•							•												•	•
<i>Xenopsylla astia</i>				•	•	•		•		•											•	•

A1- Ponnai, A2- Poigai, A3- Annaicut, A4- Munjurpet, A5- Lalapet, A6- Vellore Town, A7- Nowlok Thoppu
 Sm- *Suncus murinus*, Rr- *Rattus rattus*, Mm- *Mus musculus*, Ti- *Tetera indica*, Bb- *Bandicota bengalensis*
 R- Reference, #- Present survey, ##- Remarks, FR- First Record

Discussion

This present study was proposed to explore the ectoparasites fauna in the Vellore District of Tamil Nadu, India, an area endemic for scrub typhus in Tamil Nadu. This study was conducted during one full year and the locations were chosen as per the scrub typhus report. We could demonstrate the rodent-associated ectoparasites present in all the study sites. Earlier studies have documented more than 3000 species of chiggers all over the world and over 400 mite species were recorded in China (16). A total of 274 species of mites were collected from Yunnan Province, southwest China (16). Fujian Province documented 53 species of chiggers, Hubei Province recorded 41 chigger mite species and Beijing recorded 7 species of chigger mites (16). In India so far 204 chigger mite species were recorded. This study recorded more than 10 % of the total collection of ectoparasites from India. In this study, Vellore District situated in Tamil Nadu state is 209 times smaller than Yunnan Province, southwest China; but showed 23 species of chiggers which clearly showed that this area has a great species diversity of chigger mites. This collection has even outnumbered the collections from other investigations on ectoparasites. Total number of the vertebrate host species play very significant factor to influence the species diversity of chigger mites from different places.

A total of 11,883 chiggers were collected comprising of 65 species in 14 genera recorded from Himalayan Region (17) which includes 5 genera *Leptotrombidium*, *Neotrombidium*, *Ascoschoengastia*, *Schoengastia* and *Schoengastiella* collected from the present Vellore study. During 1966, *Leptotrombidium akamushi*, *Leptotrombidium delicense*, and *Schoengastiella ligula* were collected from Eastern Himalayan foothills (18). Except for *Leptotrombidium akamushi*, the other 2 species of mites were collected in our study. A survey conducted between 1966 and 1970 in Western Himalayas, Sikkim and Hill districts of West

Bengal showed 66 species of Ixodoidea, 15 species of Mesostigmatid mites and a large number of Trombiculid mites in addition to other haemotophagous arthropods (19). The mite's collections reported 23 genera and 54 species belonging to 10 families from Western Himalayas, Himalayan districts of West Bengal and Sikkim between 1966 and 1970 (20).

In the worldwide distribution pattern of acarines approximately 12% of 50,000 live as the parasites and less than 100 species are reported to have medical and veterinary importance (21). Many acarines act as the vectors of zoonotic diseases and live very close to the animal host. Moreover, environmental degradation led to global climate changes in many places. This led to the proliferation of the vectors and in turn transmission of rodent borne parasitic diseases (3). Many ectoparasites of rodents play crucial role in the disease transmission to human and animals. Thus, this study on ectoparasites is an invaluable data to take up appropriate control programme. Medically important chigger mites, *Leptotrombidium (Leptotrombidium) deliense* and *Schoengastiella ligula* are the powerful vectors of scrub typhus. Abundant collection of these species denotes the potential risk for the transmission of this emerging disease in these areas. *Echinolaelaps echidninus* has medical importance and could induce cross-reactivity with other allergic mites (22).

Scrub typhus is caused by *Orientia tsutsugamushi* which is transmitted by an arthropod vector of the Trombiculidae family chigger mites *Leptotrombidium deliense*. This is the most common re-emerging Rickettsial infection in India and many other Southeast Asian countries. This vector is collected aplenty in this present collection. An outbreak investigation conducted in Kurseong, Darjeeling established *Schoengastiella ligula* as the vector of scrub typhus due to its abundance (23). In this study, *Schoengastiella ligula* was also collect-

ed. Redbird mite *Dermanyssus gallinae* acting as the vectors of fowl tick fever, endemic typhus, and St. Louis encephalitis was reported from this collection (24). Similarly, tropical rat mite *Ornithonyssus bacoti* was found infested on the laboratory-reared mice colony from Nilgiris, Tamil Nadu. During 2013, *Ornithonyssus bacoti* was recovered from the domestic and peri-domestic *Rattus rattus* (25). One more Rickettsial pox vector species *Liponyssoides sanguineus* was also collected from this study (22). This study reported ticks like *Rhipicephalus turanicus* which plays a potential role as bridge vector carrying tick-borne protozoan sporozoite pathogen *Hapatozoon canis* among various hosts like dogs, foxes, and golden jackals. In this study a hard-bodied tick *Haemophysalis bispinosa* belonging to the genus *Haemophysalis* was found in India. It is obligate ectoparasites of mammals and a potential vector of Kyasanur Forest disease virus. Tropical rat fleas *Xenopsylla cheopis* and *Xenopsylla astia* were also collected in this study. *Xenopsylla cheopis* and *Xenopsylla astia* are responsible for the transmission of *Yersinia pestis* the causative agent for the spread of plague and generally collected from *Rattus rattus* and *Rattus norvegicus*. The predominance of fleas was reported from Angola (26). In Indonesia, *Xenopsylla cheopis* was the most common on *Rattus rattus* (27). In Iran, fleas catch was related to *Rattus norvegicus* (28). In this present study, *Xenopsylla cheopis* and *Xenopsylla astia* were collected from both *Rattus rattus* and *Suncus murinus*. There was no flea collected from the urban site which may not be a suitable area for the survival of fleas as observed in other areas in Malaysia (3).

According to rodent host and location, the distribution pattern of the ectoparasites differed (29). In Egypt *Mus musculus* harbored less ectoparasites but the other species like *Rattus rattus frugivorus*, *Rattus norvegicus* and *Meriones shawi* were vulnerable to ectoparasites (30). In our present study, *Suncus murinus* and *Rattus rattus* were more vulnerable than the rest

of the rodent species. 348 chiggers, two fleas, one tick and two adult mites were collected from six *Suncus murinus*. Similarly, 568 chiggers, 34 fleas, three ticks and 1 adult mites were collected from 26 *Rattus rattus*. From the rest of the rodents *Tatera indica*, *Bandicota bengalensis* and *Mus musculus*, very few ectoparasites were collected.

A taxonomic checklist of ectoparasites collected from Vellore District, Tamil Nadu, India

Chigger mites

Family: Trombiculidae Ewing, 1929

Subfamily: Trombiculinae Ewing, 1929b

Tribe: Trombiculini Vercammen-Grandjean, 1960

Genus: *Leptotrombidium* Nagayo et al. 1916

Subgenus: *Leptotrombidium* Nagayo et al. 1916

1. *Leptotrombidium (Leptotrombidium) deliense* (Walch, 1922)

2. *Leptotrombidium (Leptotrombidium) dehradunense* Stan Fernandes and Kulkarni, 2003

3. *Leptotrombidium (Leptotrombidium) delimushi* Vercammen-Grandjean and Langston, 1976

4. *Leptotrombidium (Leptotrombidium) keukenschrijveri* (Walch, 1923)

5. *Leptotrombidium (Leptotrombidium) insigne* Stan Fernandes and Kulkarni, 2003

6. *Leptotrombidium (Leptotrombidium) kulkarnii* Vercammen-Grandjean and Langston, 1976

7. *Leptotrombidium (Leptotrombidium) spilletti* Mitchell and Nadchatram, 1966

8. *Leptotrombidium (Leptotrombidium) jayewickremei* (Womersley, 1952)

Subgenus: *Ericotrombidium* Vercammen-Grandjean and Andre, 1966

9. *Leptotrombidium (Ericotrombidium) rajasthanense* Stan Fernandes and Kulkarni, 2003

10. *Leptotrombidium (Ericotrombidium) indicum* Stan Fernandes and Kulkarni, 2003

Genus: *Trombicula* Berlese, 1905

11. *Trombicula (Trombicula) hypodermata*, Nadchatram and Traub, 1966

Genus: *Neotrombicula* Hirst, 1925

12. *Neotrombicula* spp Hirst, 1925

Tribe: Schoengastiini Vercammen-Grandjean, 1960

Genus: *Schoengastia* Oudemans, 1910a

13. *Schoengastia kanhaensis* Mitchell and Nadchatram, 1966

14. *Schoengastia* spp. Oudemans, 1910a

Genus: *Ascoschoengastia* Ewing, 1946a

15. *Ascoschoengastia indica* (Hirst, 1915)

Tribe: Gahrlepiini Nadchatram and Dohany, 1974

Genus: *Schoengastiella* Hirst, 1915

16. *Schoengastiella argalea* (Traub and Morrow, 1957),

17. *Schoengastiella ligula* Radford, 1946b

Mites

II. Family: Laelaptidae Berlese, 1892

Genus: *Echinolaelaps* Ewing, 1929

18. *Echinolaelaps echidninus* (Berlese, 1887)

III. Family: Dermanyssidae Kolenati, 1859

Genus: *Dermanyssus* Duges, 1834

19. *Dermanyssus gallinae* (De Geer, 1778)

Genus: *Liponyssoides* Hirst, 1913

20. *Liponyssoides sanguineus* (Hirst, 1914)

Ticks

IV. Family: Ixodidae Murray, 1877

Genus: *Haemophysalis* Koch, 1844

21. *Haemophysalis (Kaiseriana) bispinosa* Newmann, 1897

Genus: *Rhipicephalus* Koch, 1844

22. *Rhipicephalus turanicus* Pomerantzev, Matikashvili and Lotozki, 1940

Fleas

V. Family: Pulicidae Billberg, 1820

Subfamily: Xenopsyllinae

Tribe: Xenopsyllini Glinkiewicz, 1907

Genus: *Xenopsylla* Glinkiewicz, 1907

23. *Xenopsylla cheopis* (Rothschild, 1903)

24. *Xenopsylla astia* Rothschild, 1903

A taxonomic checklist of rodents/shrew collected from Vellore District, Tamil Nadu, India

Rodents

I. Family: Muridae Illiger, 1811

Subfamily: *Gerbillinae* Gray, 1825

Genus: *Tatera* Lataste, 1882

1. *Tatera indica* Hardwicke, 1807

Subfamily: Murinae Illiger, 1811

Genus: *Mus* Clerck 1757

2. *Mus musculus* Linnaeus, 1758

Genus: *Bandicota* Gray, 1873

3. *Bandicota bengalensis* Gray, 1834

Genus: *Rattus* Fischer de Waldheim, 1803

4. *Rattus rattus* (Linnaeus, 1758)

Shrew

II. Family: Soricidae Fischer, 1814

Subfamily: Soricinae Fischer von Waldheim, 1817

Genus: *Suncus* Ehrenberg, 1832

Suncus murinus (Linnaeus, 1766)

Conclusion

This is the first record of 14 different species of chiggers and three species of adult mites from Vellore District, Tamil Nadu. The results of the present surveillance provided key scientific data that identifies potential disease vectors which will help the health authorities for the development of appropriate corrective measures to prevent and control the different acari-borne zoonotic diseases in this region.

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Ethical considerations

This study was approved by the LEAC Madurai Medical college Madurai, Tamil Nadu, India (ref no. 2339/E1/5/2017 dated 23.3.2017).

Conflict of interest statement

The authors declare that there is no conflict of interests.

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