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A study on ectoparasites with special reference to chigger mites on rodents/shrews in scrub typhus endemic areas of Kerala, India

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ABSTRACT: The main goal of this investigation was to record the ectoparasites living on rodents in scrub typhus endemic areas of Kerala state, India. Rodents captured alive using Sherman and wonder traps from four diverse habitations revealed, a total of 59 rodents/shrews constituted by 5 host species from families Muridae and Soricidae. In total, 1135 ectoparasites were collected on these rodents/shrews and were identified representing 23 species from 10 genera in 4 families Trombiculidae, Laelaptidae, Ixodidae and Pulicidae. Dominant insectivore species was *Suncus murinus* (57.6%). 42 (71.2%) rodents and shrews were found to be plagued with at least one of the 23 species of ectoparasite harvested. Mites belonging to the family Trombiculidae were the predominant ectoparasite species collected. Study revealed *Tatera indica* (35.5%) as the primary host harboring the chigger mites. *Xenopsylla cheopis* and *Xenopsylla astia* the flea species important vectors for the transmission of zoonotic diseases such as *Leptotrombidium deliense*, *Schoengastiella ligula* and *Echinolaelaps echidninus* were recorded. Twenty species of mites were reported for the first time in Kerala which add knowledge on the ectoparasites distribution.

KEYWORDS: Muridae, Soricidae, Prostigmata, flea species, zoonotic diseases, vectors

INTRODUCTION

Ectoparasites like mites, fleas, ticks, and lice are hematophagous arthropods regularly found on small mammals like rats, mouse, bandicoots (rodents), and shrew (an insectivore) host. These arthropods live on the body surface of the hosts (Mullen and Durden, 2019) transmitting the arboviral diseases, typhus fevers, plague, tularemia, leptospirosis (Masan and Stanko, 2005), and some parasitic zoonoses like babesiosis to humans and animals (Gratz, 1994; Paramasvaran *et al*., 2009). The ectoparasites of rodents play a vital role in the transmission of vector-borne diseases like scrub typhus and plague. Scrub typhus is an emerging zoonotic disease and the epidemiology of the disease revealed a male predominance with clustering of cases in hilly areas of Kerala (Krishnan *et al*., 2016). Rodents and shrews that live nearby human dwellings are considered synanthropic species, having an important role in the transmission of disease to humans and domestic animals leading to economic losses to agriculture (Brown and Khamphoukeo, 2007).

In rodents and shrews, chigger mites are predominant organisms which are the larval stages of mites belonging to the Trombiculidae family.

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Among the different ectoparasites recovered from the rodents/shrews, chigger mites were the predominant ectoparasites (Sharma, 2014). During the past ten years, more outbreaks of scrub typhus occurred during winter months in northern and central parts (Chakraborty and Sarma, 2017) and also in southern parts of India particularly Kerala and Pondicherry (Stephen *et al*., 2015). The chigger mites transmit scrub typhus and some other zoonoses (Varma, 1969; Wu *et al*., 1996; Lee *et al*., 2009). Several reports of scrub typhus from various parts of India have been published (Mathai *et al*., 2001 and 2003; Somashekar *et al*., 2006; Sharma *et al*., 2005). In India, scrub typhus and other typhus fevers are caused by various ectoparasites commonly found in regions having dense vegetation where this disease was spreading fast (Kumar *et al*., 2014). During the summer and post-monsoon season, the prevalence and diversity of mites and fleas were higher (Sadanandane *et al*., 2016).

Scrub typhus was a major outbreak disease during 2017 in Kerala. Based on our earlier study the prevalence of vector mites was already reported in the scrub typhus affected areas of Krishnagiri district, Tamil Nadu (Philip Samuel *et al*., 2017). A comprehensive survey on the ectoparasites was undertaken in the scrub typhus endemic Thiruvananthapuram, Kerala to determine the information on the different ectoparasites species distribution. The present survey aimed to report the variety of ectoparasites associated with the rodents/ shrews prevalent in the scrub typhus affected areas in Thiruvananthapuram, Kerala.

MATERIAL AND METHODS

Thiruvananthapuram (Latitude 8.524139 and Longitude 76.936638) situated in the Kerala state of India is one of the 14 districts in the Kerala state. The geographical area of this district is 2,192 km^2. The total population as per the 2011 census is 3.3 million. The average annual temperature in Thiruvananthapuram is 26.7^{0}C/80.0^{0}F and the annual rainfall is 1,828 mm/ 72 inches. According to the earlier history of this disease, the maximum number of cases reported in Thiruvanathapuram was selected for this study in consultation with the local health officials. Trapping of rodents was conducted in the four habitats during 2017 to 2018. The habitats include urban area (Kollamthara-Thiruvallam PHC), coastal area (Kidarakuzhi-Vizhinjam PHC), rural area (Pavachakuzhi-Vilavoorkal PHC), and forest area (Panga-Aruvikara PHC) (Fig.1).

Sample collection: All the rodents were captured alive using Sherman traps and wonder traps. In each of the scrub typhus positive selected villages, 50 traps were set outdoors (peri-domestic areas) with scrubby vegetation and rodent burrows. Traps were set in the evening (6.00 pm) and retrieved the next day morning (7.00 am). Rodents were anesthetized for the collection of ectoparasites, (Aplin et al., 2003; Sadanandane *et al*., 2016) transported to the laboratory.

Taxonomic Identification: Identified the rodent species by taxonomical keys (Aphin *et al*., 2003; Dinesan *et al*., 2006; Cunningham and Moors, 1996). Ectoparasites were preserved in 80% ethanol, transferred to clearing agent and mounted in Hoyer's medium, examined under the microscope, and identified up to species level (Fernandes and Kulkarni, 2003; Goff *et al*., 1982; Kerans and Litwak, 1989; Nadchatram and Alexander 1974; Sharif, 1930; Geevargheese and Mishra, 2011). All collected ectoparasite specimens were preserved on microscope slides and deposited in the Mosquito Museum Entomology Laboratory of ICMR-Vector Control Research Centre, Field Station Madurai, Tamil Nadu. This study was approved by the Institutional Animal Ethics Committee (IAEC) of Madurai Medical College, Madurai. The data analysis was performed using SPSS Ver. 15 (Statistics Package for Social Sciences).

RESULTS

A total of 59 rodents/shrews constituted by five host species of rodents/shrews (*Rattus rattus*, *S. murinus*, *T. indica*, *B. bengalensis*, and *M. musculus*) were collected from four diverse habitations. In total, 1135 ectoparasites (chigger mites, ticks, and fleas) were collected from Thiruvananthapuram. These ectoparasites were

Fig.1 Locations where the field works was carried out in Thiruvananthapuram district, Kerala

identified to be 23 species from 10 genera in 4 families.5 rodents/shrews species were entrapped from the coastal areas, 3species were snared from rural areas, 4 species were trapped from urban areas and another 2 species ensnared from forest habitation. *S. murinus* (57.6%) was the dominant insectivore species collected. 42 (71.2%) rodents and shrews were found to be plagued with at least one of the 23 species of ectoparasite harvested. A total of 23 species of different species of ectoparasites comprising five main groups, mites (Mesostigmata), chiggers (Prostigmata), ticks (Acarina), fleas (Siphonaptera) and louse (Phthiraptera) were recovered from rodents and insectivores from all the four varied habitats. Chigger mites 96% (1112) were the predominant ectoparasites found on rodents and insectivores from all the four habitats followed by fleas 2.2% (13) ticks, 0.83% (7) fleas, and 0.83% (3) adult mites (Table 1 and 2). Mites belonging to the family Trombiculidae were the predominant ectoparasites species collected. Our study revealed *T. indica* (35.5%) as the primary host harboring the chigger mites. Ticks linked to the family Ixodidae were harvested mainly from the urban-dwelling insectivores. *X. cheopis* and *X. astia* was the flea species recovered.

There was no significant difference in the distribution pattern of rodents in these localities (F-0.383, df-4, P>0.05). There was also no significant difference in the sex-wise distribution of male and female rodents/shrews in the prevalence of ectoparasites (t- 1.306, df-57, P>0.05). In the insectivores, only *S. murinus* was trapped. But, insectivores were collected more than rodent counterpart. There was a significant difference regarding infestation between rodents and insectivores (t- 2.607, df-57, P<0.05). The distribution of ectoparasites in these four localities showed a significant difference (F-8.662, df-1152, P<0.05). There was no significant difference in the infestation of ectoparasites between male and female hosts trapped (F-0.707, df-1152, P>0.05). There was a significant difference between rodents and insectivores for the presence of ectoparasites (t- 2.607, df-57, P<0.05), and a significant difference was shown in the infestation of ectoparasites in rodents and insectivores (F-25.832, df-1152, P<0.05). Taxonomic checklist of ectoparasites and rodents/shrews are also listed (Table 3 and 4).

DISCUSSION

Many species of mites and ticks harbored by rodents/shrews act as the vectors and thus gained significant medical and veterinary importance (Krantz and Walter, 2009). Scrub typhus is a re-emerging zoonotic disease initially affecting rodents being transmitted to humans through the bite of infected *L. deliense* mites which are found

Table 1. Ectoparasites indices for rodents for four different habitats

Location*	No of traps fixed	No of traps positive	Trap positivity rate	No. of Rodents/ Shrews Collected	No. of rats +ve for chigger mites	Percent positive for Chigger	No. of chigger collected	Chigger Index	No. of rats +ve for fleas	Percent positive for Fleas	No. of fleas collected	Flea Index	No. of rats +ve for ticks	Percent positive for Ticks	No. of ticks collected	Tick Index	No. of rats +ve for Mites	Percent positive for mites	No. of mites collected	Mite Index
Urban	100	12	12.00	13	6	46.2	227	17.46	0	0.0	0	0	0	0.0	0	0.00	0	0.0	0	0.00
Coastal	150	22	14.67	22	14	63.6	346	15.73	1	4.5	3	0.14	1	4.5	8	0.36	3	13.6	3	0.14
Rural	100	16	16.00	16	11	68.8	286	17.88	1	6.3	4	0.25	0	0.0	0	0.00	0	0.0	0	0.00
Forest	100	8	9.00	8	8	100.0	253	31.62	0	0.0	0	0	2	22.2	5	0.56	0	0.0	0	0.00
Total	450	59	13.11	59	40	66.7	1112	18.53	2	3.3	7	0.117	3	5.0	13	0.22	3	5.0	3	0.05

*-Urban-Kollamthara, Thiruvallam, Coastal-Kidarakuzhi, Vizhinjam, Rural-Pavachakuzhi, Vilavoorkal, Forest-Panga, Aruvikara

associated with grassland, bushy areas, gardens, beaches, and forests. Similarly, infected rodents spread many zoonotic diseases indirectly to human beings through the bite of mites, ticks, or fleas. Scrub typhus cases were reported in Kerala from 2000 onwards (Ittyachen, 2009). This study monitored the prevalence of ectoparasites in Thiruvananthapuram, Kerala to unearth the presence of different disease vectors.

Infestation rate for chiggers was 67.8% followed by ticks 3.39%, fleas 3.39% and mites 1.69%. In the present study *T. indica* from urban & coastal areas, *Bandicota bengalensis* from rural areas, and *R. rattus* from forest areas harvested with more ectoparasites while *M. musculus* harbored the lowest number of ectoparasites. The study showed the distribution pattern and density of ectoparasites differed as per the distribution of rodent hosts and locations (Hi *et al*., 1999). All the ectoparasites representing chiggers, fleas, ticks, and mites are available in the coastal area. An ectoparasite prevalence study conducted in Egypt showed more ectoparasites on *R. rattus*, *R. norvegicus* and *Meriones shawi* (Kia *et al*., 2009). *Tatera indica* is a major rodent host on the rice ecosystem, was trapped at urban villages of Thiruvananthapuram, and was found to be a good source for the collection of nine species of chiggers including *Trombicula hypodermata*. *Bandicota bengalensis*, lesser Bandicoot was trapped in less number found only in the coastal area was also a good source for the collection of 9 species of chiggers. All study sites were found positive for *S. murinus* rich in chigger ectoparasites. Vector mite *L. deliense* and plague vector *X. cheopis* were already recorded from Thiruvananthapuram during 2012 (Sharma, 2013).

In the present study, *H. kumari* was recorded in shrews *S. murinus*. An Ixodidae tick species *Hyalomma anatolicum anatolicum* was already recorded in a study conducted on the domestic animals in Kerala along with the same genus *H. marginatam isaaci* and *H. hussaini* on buffalo and cow (Prakasan and Ramani 2007). An Ixodidae tick species *Hyalomma sp*. was reported from Sarpole Zehab, Kwermanshah Province, Iran (Telmmafaarraiy *et al*., 2015). Ixodid ticks are the main parasites of different domestic animals in India. Tick infection on domestic animals is a major problem for its proper development in Kerala. *Hyalomma anatolicum* is a vector for the Crimean-Congo hemorrhagic fever with Nirovirus (Bunyaviridae) as the pathogen. *Hyalomma anatolicum* also transmits protozoa of the genus Theileria (Ghosh and Nagar, 2014). Ticks were mainly collected in coastal and forest sites and all ticks were collected only from *S. murinus*. Contrary to this, no ticks were collected from the other rats in Malaysia (Paramasvaran *et al*., 2009). This is also supporting the presence of ticks in urban rodents which was reported in Malaysia (Audy and Nadchatram, 1957).

There was no flea collected from the urban and forest areas which may not be suitable for the survival of these fleas as already observed in other areas (Geevargheese, 1997). The prevalence of fleas in the coastal and rural villages of Thiruvananthapuram showed a favorable environment

Table 2. Species-wise ectoparasites recovered from field rodents and shrews trapped

Villages	Urban			Coastal				Rural			Forest		Ectoparasites				
Ectoparasites/Hosts	BB	TI	SM	BB	TI	SM	RR	SM	BB	M M	RR	SM	R1	R2	R3	PS	Remarks
Chigger mites																	
Leptotrombidium deliense	●	●	●				●	●	●	●	●	●		●		●	
L. discrepans		●					●									●	New record
L. insigne		●														●	New record
L. spilletii		●														●	New record
L. kulkarni		●														●	New record
L. delimushi	●															●	New record
L. fulmentum									●							●	New record
L. rajesthanense									●							●	New record
Leptotrombidium sp		●							●							●	
Schoengastiella pracipua	●															●	New record
S. ligula		●									●					●	New record
S. ralagea				●					●			●				●	New record
S. helata					●				●							●	New record
S. bengalensis									●							●	New record
Schoengastiella.sp									●							●	
Herpetacarus schlugeri	●			●		●		●	●			●				●	New record
Microtrombicula kanjutekrii		●			●											●	New record
M. khurdagencosa					●											●	New record
Neotrombicula fujigmo	●															●	New record
N. microti					●											●	New record
Neotrombicula sp.		●														●	
Trombicula hypodermata	●				●						●					●	New record
Walchia rustica									●							●	New record
Mites																	
Echinolaelaps echidninus						●										●	New record
Ticks																	
Hyalomma kumari						●						●	●			●	
Fleas																	
Xenopsylla cheopis				●										●	●	●	
X. astia										●					●	●	

SM-*Suncus murinus*, RR-*Rattus rattus*, MM-*Mus musculus*, TI-*Tatera indica*, BB -*Bandicota bengalensis,*R1-(Geevarghese *et al*., 1997), R2-(Sharma, 2013), R3-(Sharif, 1930). PS - Present survey

Table 3. Taxonomic checklist of ectoparasites collected from the rodents/shrews

Family/ Subfamily	Tribe	Genus	Subgenus	Species
Trombiculidae Ewing, 1929/ Trombiculinae Ewing, 1929b	Trombiculini Vercammen-Grandjean, 1960	*Leptotrombidium* Nagayo et al., 1916	*Leptotrombidium* Nagayo et al., 1916	*deliense* (Walch, 1922) *insigne* Stan Fernandes & Kulkarni, 2003 *delimushi*Vercammen-Grandjean & Langston, 1976 *discrepans* Fernandes, 1988. *fulmentum*Vercammen-Grandjean & Langston, 1976 *kulkarnii*Vercammen-Grandjean & Langston, 1976 *spilletti* Mitchell & Nadchatram,1966
			Ericotrombidium Vercammen-Grandjean & Andre, 1966	*rajasthanense,* Stan fernandes and Kulkarni, 2003
		Trombicula Berlese,1905		*hypoderma*, Nadchatram and Traub, 1966
	Schoengastiini Vercammen-Grandjean, 1960	*Neotrombicula* Hirst, 1925		*fujigmo* (Philip and Fuller), 1950
				microti (Ewing) 1928
		Microtrombicula Ewing, 1950	*Microtrombicula* Traub & Nadchatram, 1966a	*kajutekrii* (Joshee, 1964) *khurdagencosa* Fernandes, 1988
		Herpetacarus Vercammen-Grandjean, 1960	*Herpetacarus* Vercammen-Grandjean, 1960	*schlugeri*(Radford), 1953
	Gahrliepiini Nadchatram & Dohany, 1974	*Schoengastiella* Hirst,1915		*bengalensis* Hirst, 1915 *helata* (Traub and Evans), 1954 *ligula* Radford, 1946b *praecipua* Nadchatram and Fernandes, 1989 *ralagea* Fernandes, 1988
		Walchia Ewing, 1931		*rustica* (Gater 1932)

present for its survival. Generally, fleas are collected from *R. rattus* and *R. norvegicus* as already reported from Angola (Linardi *et al*., 1994). In Indonesia, *X. cheopis* was the most common on *R. rattus* (Durden and Page, 2008). In Iran, the fleas catch was related to the availability of *R. norvegicus* (Kia *et al*., 2009). In the present study, *X. cheopis* was collected only in *B. bengalen-*

Table 4. Taxonomic checklist of rodents/shrews collected

Family	Subfamily	Genus	Species
Rodents - Muridae Illiger, 1811	Gerbillinae Gray, 1825	*Tatera* Lataste, 1882	*indica* Hardwicke, 1807
	Murinae Illiger, 1811	*Mus* Clerck, 1757	*musculus* Linnaaeus, 1758
		Bandicota Gray, 1873	*bengalensis* Gray, 1835
		Rattus Fischer de Waldheim, 1803	*rattus* (Linnaeus, 1758)
Shrew - Soricidae Fischer, 1814	Soricinae Fischer von Waldheim, 1817	*Suncus* Ehrenberg, 1832	*murinus* (Linnaeus, 1766)

sis from coastal area and *X. astia* in *M. musculus* recorded from rural areas. Fleas were collected from *R. norvegicus*, *R. rattus,* and Hamster in Bandar Abbas, Southern Iran (Kia *et al*., 2009). No lice were collected in the Thiruvananthapuram area from the rodents collected from the areas. The available rodents from these places, considered as infected, were *M. musculus* spreading 14 different diseases, *R. rattus* spreading 13 different diseases and *T. indica* spreading 4 different diseases and *S. murinus* spreading one disease (Rabiee *et al*., 2018; Hi *et al*., 1999). *B. bengalensis* can also cause a range of diseases and cause very similar concerns as the other commensal rats. *Rattus rattus* (common house rat) cause the spread of diseases like plague, typhus & leptospirosis and is a serious pest in Kerala (Dinesan *et al*., 2006).

In this study, medically important ectoparasites like *L. deliense* (scrub typhus vector), *S. ligula* (Scrub typhus vector) Tilak *et al*., 2011), *E. echidninus* (induce cross-reactivity with other allergic mites (WHO, 1986; Kia *et al*., 2009), *X. cheopis*, and *X. astia* (plague vectors) (Shashi *et al*., 2013) were recorded which showed the potential risk for the transmission of zoonotic diseases. Thiruvananthapuram climatic condition favored the development of the ectoparasites and different species of rodents/shrews (Saxena 1989; Kumar *et al*., 2004). The proliferation of these acarine vectors will increase the contact between human and rodents which in turn promote the transmission of various acari-borne zoonotic diseases and enhance the disease burden.

This study reports nineteen species of chigger mites and one species of adult mite for the first time report in Kerala. All of the surveyed areas are receptive to the high risk of transmission of scrub typhus. Rodent/shrew, chigger vector mite, and flea control are also to be undertaken to prevent the further spread of the diseases by these vectors. Information, education, and communication (IEC) awareness campaigns are to be taken up to sensitize the public about these diseases. Different risk factors like epidemiological, behavioral, and environmental risk factors about these areas are to be identified and appropriate measures are to be taken up to sensitize the public. In the coastal area rodents/shrews had a higher ectoparasitic infestation. The current knowledge on ectoparasites load on rodents in Kerala brings forth the main reason for the sudden resurgence of the scrub typhus cases in many places. Monitoring of rodent population and their ectoparasites brings forth important data to facilitate arthropod-borne disease control strategies by the public health authorities.

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