https://doi.org/10.33307/entomon.v47i2.717
ENTOMON 47(2): 165-170 (2022)
Short communication No. ent. 47210

Cross sectional studies on the ectoparasites among rodents in scrub typhus cases in Karnal and Kaithal Districts of Haryana, India

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ABSTRACT: *Orientia tsutsugamushi* is a mite-borne bacterium belonging to the family Rickettsiaceae and is responsible for a disease called scrub typhus in humans, which is transmitted by the vector mite *Leptotrombidium deliense* (common ectoparasite on rodents) in most of Asian countries including India. The study conducted in selected villages of Karnal and Kaithal districts of Haryana state, India revealed four species of rodents - *Rattus rattus, R. norveigicus, Bandicota indica* and *Suncus murinus.* Dust mite *Dermatophagoides farina*; chigger mite *L. deliense and* fleas *Xenopsylla astia* and *X. cheopis* were prevalent on the rodents.

KEY WORDS: Vector mite, chigger mite, dust mite, flea, rickettsial disease

Scrub typhus is a rickettsial disease caused by *Orientia tsutsugamushi* a mite-borne bacterium belonging to the family Rickettsiaceae. It is transmitted to humans by the bite of infected vector chigger mites. The trombiculid chigger mites are common ectoparasites on rodents and belong to the genus *Leptotrombidium* (Acariformes: Trombiculidae). Of these, the most common are *L. pallidum* (Nagayo), *L. deliense* (Walsh), *L. scutellare* (Nagayo) and *L. akamushi* (Brumpt) (Acosta-Jamett *et al.*, 2020). Historically, scrub typhus had been endemic in Asia, Australia, and islands in the Indian and Pacific Oceans, known as the "tsutsugamushi triangle" (Bonell *et al.,* 2017). However, there have been recent reports of scrub typhus from Africa, the Middle East, and South America suggesting that the disease is no longer restricted to this triangle (Jiang and Richards, 2018). But no indigenous cases have been reported from North America and Europe. Scrub typhus is frequently reported from many Asian countries and is endemic in Nepal and its neighboring countries including India (Sub-Himalayan belt) and Bhutan, where it is considered an emerging infectious disease (Jeromie Wesley Vivian, 2017; Ranjan and Prakash, 2018; Tshokey *et al.,* 2018). Now it is re-emerging in almost in all states of India (Tilak and Kunte, 2019). The other vector mite spe-cies

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detected based on molecular techniques are *Ascoschoengastia indica* (Hirst), *Guntheria cassiope* (Womersley), *Odontacarus* sp. (Ewing), *Eutrombicula wichmanni* (Oudemans), and *Microtrombicula chamlongi* (Nadchatram and Kethley) (Elliott *et al.*, 2019). These mites contribute for the transmission in different regions of India (Ranjan and Prakash, 2018). Knowledge of the vector, including species, distribution, density, and habitats, is important to understand the epidemiology of scrub typhus in a given area or region (Park *et al.*, 2016). Clinical scrub typhus cases were reported in the districts of Karnal and Kaithal of Haryana state and most of them were confirmed in the laboratory of the PGIMER, Chandigarh. This study was undertaken with the main objective in finding out the vector mites among rodents that takes part in transmission of scrub typhus in the villages.

Study site: Haryana State has been contributing more number of scrub typhus cases from Karnal, Kaithal, and Panchkula districts with reference to the monthly periodical reports of National Centre for disease control (NCDC), Delhi, Based on that, a cross sectional entomological investigation was made in Sagger ($29^0$41’8.2644’’N; $76^0$59’25’’E) and Dadapur (28^0N; 76.6629^{0}E) villages of Karnal District; Pundri (29.7621^{0}N; 76.5546^{0}E) and Harsola ($29^0$48’5.51"N; $76^0$23’58.52"E) villages of Kaithal District of Haryana State. The GPS locations of areas from where rodents were trapped. Sherman and Wonder’s traps were used. Single trap was placed in the scrub typhus positive house and two traps in the neighboring houses in each village. The sources of rat dwelling were identified by the presence of burrows, their paws, and excreta. Traps were laid at 6 PM and were collected in the next day morning by 6 AM (12 hours). A piece of Chapatti and roasted coconut were used as rodent baits. Rodents were identified using morphometric characteristics (Agrawal, 2000). Chigger mites were gathered from the rodents after they were euthanized using chloroform (Sigma–Aldrich, Bangalore, India) as described previously (Park *et al*., 2016). The chiggers were identified using the standard key for iden-tification of Indian *Trombiculidae* (Fernandes and Kulkarni, 2003). The identification features included the shape of the scutum, specialized leg setae, palpal chaetotaxy and chelicerae (Kuo *et al.*, 2015, Kumlert *et al.*, 2018; Philip *et al.*, 2021). The confirmation of the genus and species were based on pub-lished keys (Fernandes and Kulkarni 2003; Philip *et al.*, 2021). The dust mites were identified using the pictorial keys (Calloff and Stewart, 1997).

About 500g of soil and litter (humus samples) in and around rat burrows was collected and packed in zipped sachets and brought to the laboratory for detection of chigger mites employing Berlese’s Funnel method. Berlese funnels are used for extracting the arthropods from soil and litter samples (Philip *et al.,* 2021). The organisms were identified using a binocular microscope (Dewinter) at 100X magnification. The Indices have been computed adopting the following standard formulae (Philip *et al*., 2021; Basker *et al*., 2022).

Chigger index: It is exclusively for Mite Borne Disease. It is measured by number of chiggers infested by a single host.

Prevalence rate of mites: Number of Hosts with ectoparasites / Total number of hosts examined

Mean intensity of ectoparasites from host animals: Total number of ectoparasites collected / Number of hosts infected with ectoparasites

Total flea index = Total number of fleas collected (regardless of species), divided by the total number of hosts examined.

Among the 12 traps laid at villages, viz., Sagger, Dadapur of Karnal District, and Pundri and Harsola villages of Kaithal District, six traps successfully trapped rodents. Four species of rats were encountered viz., *Rattus rattus, R. norveigicus, Bandicota indica and Suncus murinus*. Four species of ectoparasites isolated from the rats were - *Dermatophagaoides farinae, Leptotrombidium deliense* and fleas, *Xenopsylla astia* and *X. cheopis.* A total of 39 ectoparasites of all species could be collected. Among the mites, *D. farinae* was more prevalent (22 nos. and 56.4%) in rodents

Table 1 Prevalence of ectoparasites among the rodent species

Village	Rodents/ source	Ectoparasites - no./ species/(%)
Sagger	*Rattus rattus*	1 *Dermatophagoides* spp (4.55%)
Dadapur	*Bandicota indica*	17 *Dermatophagoides* spp (77.27%)
		5 Trombiculid spp (33.3%)
Dadapur	*Suncus murinus*	6 *Trombiculid* spp (40%)
Pundri	*R. rattus*	1 female *Xenopsylla cheopis*
		1 male *X. astia*
		3 *Trombiculidae* spp (33.3%)
		2 *Dermatophagiodes* spp (9.09%)
Harsola	*R. norveigicus*	1 *Trombiculidae* spp (6.7%)
		2 *Dermatophagoides* spp (9.09%)
Dadapur	soil and litter	2 *Dermatophagiodes* spp.

Table 2. Ectoparasites and their indices in scrub typhus reported villages in Karnal and Kaithal

Village (District)	Rodent	Dust mites – no./ (%)/ Index	Chiggers spp no./ (%) / Index	Fleas no./ Index	Prevale-nce rate	Mean intensity
Sagger (Karnal)	*Rattus rattus*	1 - (4.54%) - 0.5	0 - 0 - 0	0 - 0	0	0
Sagger (Karnal)	*Suncus murinus*	0	0 - 0 - 0	0 -0	0	0
Dadapur (Karnal)	*Bandicota indica*	17 - (77.27%) - 17	5 - (33.3%) - 5	0 -0	1	14
Dadapur (Karnal)	*S. murinus*	0	6 - (40%) - 6	0 - 0	1	14
Pundri (Kaithal)	*R. rattus*	2 - (9.09%) - 2	3 - (20%) - 3	2* - 1	1	7
Harsola (Kaithal)	*Rattus norveigicus*	2 - (9.09%) - 2	1 - (6.7%) -1	0	1	3

*1 female *Xenopsylla cheopis* and1 male *X. astia* flea

followed by chigger mite *L. deliense* (15 nos. and 38.4%) and species of fleas (2 and 5.1%). All the species were found on the rodents. From the soil and litter sample extraction *D. farinae* (2 no.) could be recorded (Table 1).

Chigger trombiculid mites, *L. deliense* were infested maximum on *S. murinus* (40%) followed by in *B. indica (*33.3%), *R. rattus* (20%) and *R. norveigicus* (6.7%*)*. Dadapur of Karnal, was identified as more vulnerable for scrub typhus. *D.*

farinae was more detected in Dadapur and most of them were infesting *B. indica*. The soil sample collected in and around the rodent burrows from Dadapur village also showed the presence of *D. farinae*. The chigger index of Dadapur was 5.5; in Pundri, chigger index was 3 and in Horsala of Kaithal district, it is 2. Among these villages, Dadapur showed higher chigger index. Since these indices are greater than the critical chigger index of 0.69 (Olson *et al.*, 1979; Basker *et al.*, 2022), the probability of scrub typhus transmission in these villages is more. The prevalence rate of mite is one for all the rodent species. Mean intensity of ectoparasites from host animals is 14 each for *B. indica* and *S. murinus.* Of the fleas detected there were both the primary and secondary vectors of plague and were isolated from *R. rattus* which was captured in the semi-urban of Kaithal. Mean ectoparasites present per animal is dust mites 3.66, chigger mites 2.50 and fleas 0.33 (Table 2).

The bacteria *O. tsutsugamushi* cause scrub typhus and is transmitted by *Leptotrombidium* mites. It is responsible for a potentially fatal tropical infection which is a grossly under-recognized public health problem in India (Bonell *et al.,* 2017; Behera *et al.,* 2019). This disease is known to occur in diverse ecological settings in India with large numbers of cases being reported from Tamil Nadu, Andhra Pradesh, Karnataka and Kerala in the South, Himachal Pradesh, Uttaranchal, Jammu and Kashmir in the North, Meghalaya, Assam and Nagaland in the North-East, West Bengal and Bihar in the East and Maharashtra and Rajasthan in the West (Xu *et al*., 2017; Philip *et al.,* 2021).

Dust mites feed mostly on dead skin and hairs shed from humans. In Haryana state, dust mites *Dermatophagoides pteronyssinus* and *Chyletus malaccenus* were previously reported (Voorhorst *et al.,* 1969). House dust mites, especially certain species of pyroglyphids, are the cause of allergic reactions of the respiratory tract (asthma and rhinitis). In recent years, house dust mite allergy has been identified as a frequent cause of asthma, especially among children (Traub and Wisseman Jr, 1968).

Rodents are not only the reservoir of *O. tsutsugamushi* the causative organism for scrub typhus but also known to transmit human and animal diseases such as *Leptospira* spp, *Borrelia* spp., *Yersinia pestis,* and *Bartonella* spp. (Raharivolona and Ganzhone, 2009). In the present study, the potential primary and secondary plague vectors in India *X. cheopis* and *X. astica* have been isolated from the *R. rattus* which was captured from Pundri semi-urban of Kaithal district, Haryana and these species are potential enough to enhance the spread of plague within the communities in future if an outbreak occurs (Eisen *et al.,* 2014). It is imperative that high priority be given to the research and development of effective integrated rodent management programs against domestic, peri-domestic, and sylvatic rodent species to reduce the chances of parasite transmission. Since dust mite and chigger mite infestation found in rodents and soil, the surveillance on morbidity related to scrub typhus, allergy and other complications in the human community is highly essential.

ACKNOWLEDGMENTS

Authors are very grateful to the Director, National Center for Disease Control (NCDC), Delhi for providing the facilities for conducting the study. Authors thank the State Health authorities, Epidemiologists, Entomologists, Biologists, Medical Officers of PHCs, and Multipurpose Health workers of both the districts of Karnal and Kaithal districts of Haryana State, India for their help rendered during the rodent survey and interaction with the community of villages. Above all, thank Dr. Tanzin Dikid, HoD, Department of Epidemiology and HoD i/c of the Center for Medical Entomology and Vector Management, Delhi is gratefully acknowledged for utilizing the laboratory for processing and identification of arthropod samples isolated from rodents with the valuable assistance of Mr. P. Chandrasekaran.

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(Received February 17, 2022; revised ms accepted May 29, 2022; published June 30, 2022)