

Short Communication

**LEPTOTROMBIDIUM DELIENSE INFESTATION IN DOMESTIC DOGS FROM INDIA,
A VECTOR OF SCRUB TYPHUS: A CASE REPORT**

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ABSTRACT: Scrub typhus is a vector-borne, zoonotic disease caused by *Orientia tsutsugamushi*. Several members of the genus *Leptotrombidium* have gained importance due to their potential role as vectors as well as reservoirs for *O. tsutsugamushi*. The larvae of *Leptotrombidium* species are primary parasites of ground-dwelling rodents. However, changes in climate, host specificity makes them to adapt to other animals and play a role in the perpetuation of various (re)-emerging pathogens between animals and humans. Two male mongrel dogs aged six months were presented to the College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Mizoram, India with a history of skin lesions and intense pruritus. Routine skin scraping examination of samples revealed the presence of *Leptotrombidium deliense* larvae. Considering the public health importance of *L. deliense* infestation, an attempt was made to screen the dogs for *O. tsutsugamushi* and other haemoproteoans. Microscopic and molecular tests were negative for haemoproteoan parasites and *O. tsutsugamushi*, respectively. Both the dogs were successfully treated with parenteral ivermectin and topical fipronil spray.

Key words: *Leptotrombidium deliense*, Scrub typhus, Dogs, India, *Orientia tsutsugamushi*.

Trombiculosis is the infestation caused by the larval stages of ectoparasites from Trombiculidae family. Trombiculids, also known as berry bugs, scrub-itch mites, harvest mites, or chigger mites, are generally primary parasites of ground-dwelling rodents. However, due to climatic variations and other ecological factors, several infestation cases have been reported in other vertebrate animals like deer, cat and humans from different regions of the world (Santibáñez *et al.* 2015, Ramilo *et al.* 2019). *Leptotrombidium deliense* is a species of chigger mite

belonging to the family Trombiculidae. The life cycle of *L. deliense* consists of 7 stages (Fig. 1); only the larval stage is parasitic on warm-blooded mammals (Lv *et al.* 2018). This chigger mite acts as a potent vector for zoonotic Gram-negative bacteria *Orientia tsutsugamushi*, which causes scrub typhus, the second most common febrile disease next to malaria that is posing a serious public health concern in many parts of the Asia-Pacific regions, threatening nearly one billion people globally with one million new cases being reported annually (Xu

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et al. 2017, Lv et al. 2018, Crecelius and Burnett 2020). In India, several states have encountered outbreaks of scrub typhus and its associated mortality in humans (Gill et al. 2020, Banerjee and Kulkarni 2021, Manjunathachar et al. 2021). Increase in vector population is directly related to the increase in the number of scrub typhus cases. Furthermore, high rainfall and humidity provide a conducive climate for vector propagation in Northeast India.

Mizoram is a Northeast state of India which falls within the high rainfall zone and the climate varies from subtropical to alpine. The average state rainfall is 254 cm per annum (<https://des.mizoram.gov.in/uploads/attachments/08c05856c0c242a40870ed28fdadc342/pages-200-meteorological-data-of-mizoram-2020-converted.pdf>). Scrub typhus, a vector borne disease is considered as one of the major public health issues in the state due to its associated mortality in humans (Lalrinkima et al. 2017, Lalchandama 2018, Lalthazuali et al. 2020). To the best of our knowledge upon literature search, there are no reports of *L. deliense* infestation in dogs from India. Hence, we report for the first time an infestation of *L. deliense* in domestic dogs from Mizoram, India. With an interest, an attempt was made to screen the dog samples to explore any host role of dogs in relation to the occurrence of scrub typhus in human beings.

The study

Clinical examination and sampling of animals

A dog owner aged 30 years with a family size of five members having history of fever for 3-4 days presented two male mongrel dogs aged six months to the Teaching Veterinary Clinical Complex, College of Veterinary

Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, India. The dogs were presented with a history of skin lesions along with intense pruritus for three weeks. Similar clinical signs had been noticed previously in October and November before presentation. Physical examination of animals revealed the presence of crusts, erythematous, papular and pustular lesions on the body. The lesions were more pronounced on the dorsal aspect of head, ventral abdomen, inguinal region and limbs (Fig. 2). Superficial and deep skin scrapings were done from lesions with the help of scalpel blade and the wandering mite larvae on animal body were collected. Approximately 4.0 mL of blood sample was collected from each dog by venipuncture of the cephalic vein for molecular and haematological examinations. The blood with anticoagulant was used for haemogram and deoxyribonucleic acid (DNA) extraction. The blood without anticoagulant was allowed to clot and centrifuged at 2000 rpm for 10 min at 4°C to separate serum, and the serum was used for the analysis of biochemical parameters. As a routine examination, vital parameters of the animals were also recorded. Since the dog owner was suffering with fever, she was advised to visit physician to rule out the possibility of scrub typhus.

Microscopic and molecular analysis

The skin scraping samples collected were processed with 10% potassium hydroxide (KOH) and observed under the microscope for presence of external parasites (Pereira et al. 2012). The microscopic examination of skin scraping samples revealed the presence of trombiculid mite larvae. The larvae were confirmed as *L. deliense* based on morphological characteristic traits such as oval-shaped idiosoma, red ocelli on each side of rectangular scutum and five plumose setae on scutum (Fig. 3) as described earlier (Lv et al. 2018). The peripheral blood smears were prepared on clean grease free glass slides and fixed with methanol for 1 min. The smears were stained with Giemsa stain as per the standard procedure and examined under oil immersion objective for haemoprotozoan parasites (Jain 1986).

The collected mite larvae (twenty five) from two dogs were pooled and made into five samples for downstream process. Genomic DNA was extracted from pooled mite samples and blood samples of dogs using DNA Sure Mini Kit (GENETIX, India) as per the manufacture's protocol with slight modifications. Amplification of 56 kDa gene of *O. tsutsugamushi* was carried out by using two sets of primers. For primary polymerase chain reaction (PCR), forward- 5'ATTGCTAGTGCAATGTCTGC3' and

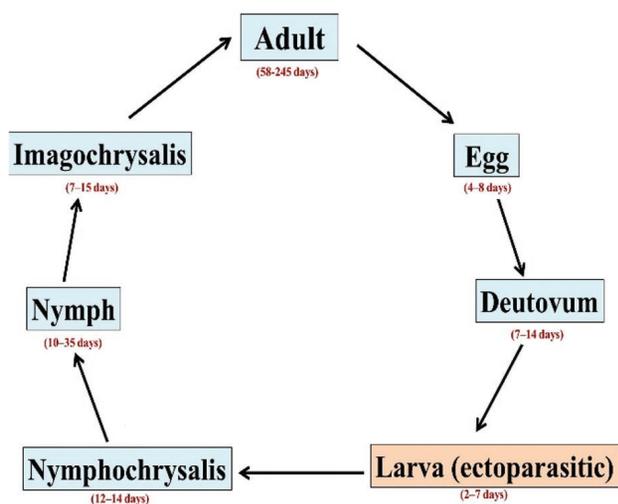


Fig. 1. Seven stages in the life cycle of *Leptotrombidium deliense* (Lv et al. 2018).

reverse- 5'CTGCTGCTGTGCTTGCTGCG3' primers pair was used. For secondary PCR, forward- 5'CCTCAGCCTACTATAATGCC3' and reverse- 5'CGACAGATGCACTATTAGGC3' primers pair was used. The primary PCR was carried out in a 20 µL reaction mixture containing 5 µL template, 2 µL of 10X Taq Buffer, 2 mM MgCl₂, 4 mM of dNTPs mixture, 0.4 µL each 10 pmol/mL primer (forward and reverse primer), 1 U of Taq DNA polymerase (Bangalore Genei, India) and the volume was made up to 20 µL by adding nuclease free water (Manjunathachar *et al.* 2021).

Haematological and serum biochemical analysis

Haematological parameters such as total erythrocyte count (TEC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), total leukocyte count (TLC) and total platelet count were measured by using a semi-automated blood cell counter (MS4-e, France) as per manufacturer's instructions. Differential

leukocyte count (DLC) was performed manually as per the method described earlier (Jain 1986). Serum biochemical parameters such as total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine were estimated as per manufacturer's instructions by using commercially available kits (Coral Clinical Systems, Goa, India). Serum globulin level was derived from the serum total protein and serum albumin concentrations using Fried-Wald's equation. Both haematological and serum biochemical parameters were evaluated before and after the treatment period, and values were compared.

Analysis of the results

Trombiculosis is a neglected and under-reported ectoparasitosis characterized by intense pruritic dermatitis. In the present study, both the dogs were showing severe signs of pruritic dermatitis and the microscopic examination of skin scraping samples revealed the presence of *L. deliense* larvae (Fig. 3).



Fig. 2. Day 0: Presence of crusts, erythematous, papular and pustular lesions on head, ventral aspect of abdomen, inguinal region and limbs, Day 28: Completely healed skin lesions on the head, ventral aspect of abdomen, inguinal region and limbs.

Around 25 larvae were harvested from two dogs. Six legged larvae are responsible for the several clinical signs such as acute dermatitis with intense irritation (Giannouloupoulos *et al.* 2012). In trombiculid mite infested animals, erythema, macules, papules, vesicles, wheals, scales, crusts and nodules with or without pruritus are typically found in body areas (head, ear pinnae, ventral abdomen, perineum and legs) which are in close contact with soil and vegetation (Ramírez *et al.* 2009, Giannouloupoulos *et al.* 2012).

Surveillance of sentinel animals to detect and monitor the circulation of pathogens in the environment and other biological threats is a globally established method (Halliday 2010). Rodents are used as a sentinel animal for few important diseases including plague. Dogs own a special place as pets and companion animals, both in developed and developing countries and they share the same environment with owners, co-exposed to the same arthropod vectors and pathogens. Canine seroprevalence studies are easier to conduct than rodent studies because dogs live in close proximity to humans, are easy to identify and access, and sampling is simple and can be replicated for follow-up studies. Hence, dogs can be used as suitable sentinel animals for the detection of zoonotic pathogens, such as the plague pathogen (Halliday 2010, Hilborn and Beasley 2015, Neo and Tan 2017). Recently, Tay *et al.* (2013) demonstrated that the owners who have close contact with dogs and other pets were more likely to be exposed to the *O. tsutsugamushi* and concluded that dogs may serve as transport hosts for infected chigger mites, increasing the risk of scrub typhus in their owners. Following confirmation of *L. deliense* infestation in dogs, the blood samples were screened for haemoprotozoan parasites and *O. tsutsugamushi*. The pooled mite samples were also processed for the detection of *O. tsutsugamushi*.

Microscopic and 56 kDa gene-based PCR tests were negative for haemoprotozoan parasites and *O. tsutsugamushi*, respectively. In addition to this, the dog owner and family members were also negative for anti-*Orientia* IgM antibodies using a rapid immunochromatographic test (ImmuneMed scrub typhus rapid test kit, South Korea) as well as for IgM ELISA (InBios International, Inc.). Dogs are also susceptible to *O. tsutsugamushi* infection and their suitability to gain insight into human exposure has been suggested in previous reports from endemic areas in Asia (Huxsoll *et al.* 1977, Nanayakkara *et al.* 2013). Because of their susceptibility, dogs may play an important role in *O. tsutsugamushi* life cycle (Namikawa *et al.* 2014). India is considered as one of the key hotspots in the 'tsutsugamushi triangle' (Lalrinkima *et al.* 2017, Elliott *et al.* 2019). Hence, in order to clearly depict the role of dogs in scrub typhus epidemiology, planned comprehensive investigations are needed, particularly in endemic states of India.

The haemogram revealed slight eosinophilia in both affected dogs while the serum biochemical parameters were all within normal range. Eosinophilia in affected dogs could be due to hypersensitivity reaction to the mite. Chigger mites induce various degrees of inflammatory reactions in the form of hypersensitivity and bacterial infections in affected animals and humans (Giannouloupoulos *et al.* 2012). The larva of *L. deliense* attaches to the moist skin of the host by means of its sharp mouth parts (chelicerae) and develops a characteristic feeding tube (stylostome) to feed on lymph, tissue fluid and blood of host (Lv *et al.* 2018, Elliott *et al.* 2019). Clusters of allergens including trypsin-like serine protease, papain-like cysteine protease, enolase have been found in *L. deliense* which may be associated with pruritic dermatitis and eosinophilia (Dong *et al.* 2018).

The dogs were treated with parenteral ivermectin at 200 µg/Kg body weight, subcutaneously, once in every two weeks; topical fipronil spray, once in a week; omega 3 and omega 6 fatty acids syrup at 5 mL/10 kg body weight, orally, twice a day, for four weeks; chlorhexidine gluconate spray (0.25% w/v) for topical application, twice a day; and cetirizine at 0.5 mg/Kg body weight, orally, twice a day, for one week. Moreover, the owner was advised to keep the dogs confined to a clean indoor environment. Dogs showed improvement in the condition after one week of therapy. The complete recovery from skin lesions was noticed after four weeks and the subsequent skin scrapping examination was negative for *L. deliense* infestation. Two more skin scrapping

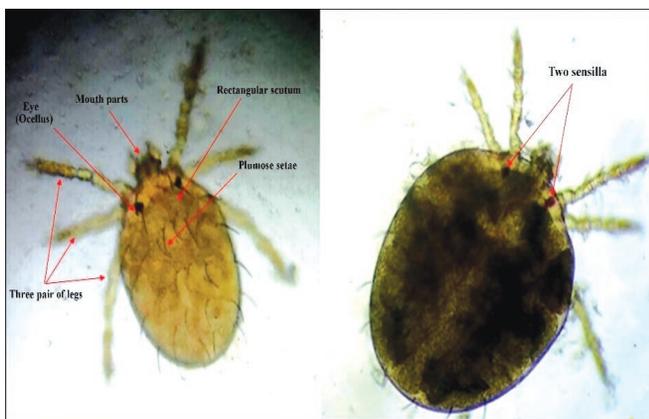


Fig. 3. Demonstration of *Leptotrombidium deliense* larvae by microscopic examination of skin scraping samples.

examinations were performed at a two weeks interval to rule out *L. deliense* re-infestation. The following regime of treatment was undertaken due to recurrence of pruritus and symptoms of allergic dermatitis in dogs. Ivermectin and fipronil have been used successfully in the management of trombiculid mite induced dermatitis in dogs and cats (Giannouloupoulos *et al.* 2012, Ramilo *et al.* 2019, Sharun *et al.* 2019). The dogs were supplemented with polyunsaturated fatty acids (omega 3 and 6 fatty acids), which help to preserve cell membrane fluidity, flexibility and functionality, and are required for the biosynthesis of intercellular lipids in the stratum corneum layer of skin (Thakur *et al.* 2019). Cetrizine was given to combat the hypersensitivity reaction due to larval feeding.

To the best of our knowledge, this is the first report of *L. deliense* infestation in domestic dogs from India. Since this was only a preliminary investigation and India is one of the hot spots under 'tsutsugamushi triangle', further studies are essential to explore the implications of our findings and to find the role of dogs as transport or sentinel animals for *O. tsutsugamushi* pathogen in India.

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