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BRIEF REPORT

Novel Vector of Scrub Typhus in Sub-Antarctic Chile: Evidence From Human Exposure

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The exposure of a research team to chigger mites in southern Chile allowed the first identification of a trombiculid species as vector and reservoir of scrub typhus outside the tsutsugamushi triangle, providing unique insights into the ecology and transmission of this recently discovered rickettsial infection in South America.

Keywords. scrub typhus; *Orientia* species; trombiculid mite; trombiculiasis; *Herpetacarus antarctica*.

Scrub typhus is a potentially severe rickettsiosis caused by *Orientia* species with a wide geographical distribution, including the tsutsugamushi triangle in Asia-Pacific, Chile, the Arabian Peninsula, and probably parts of Africa [1]. In the Asia-Pacific region the infection is transmitted by larvae of trombiculid mites, known as chiggers. In South America, endemic scrub typhus was first described on Chiloé Island in southern Chile; since then, it has been recognized in various parts of Chile, ranging over 1900 km from the Biobío Region in the North to Tierra del Fuego in the South [2–4]. The disease is caused by the proposed novel species *Candidatus* Orientia chiloensis [5], which has recently been cultured from clinical samples (C Martínez-Valdebenito, 2020, unpublished observations). The first vector studies on Chiloé Island identified 3 rodent-associated mite species in probable hot spots of scrub typhus [6]. Two species were previously unknown; one of them, *Herpetacarus eloisae*, was found to be infected with *Orientia* spp., suggesting its role as a vector of scrub typhus [7]. However, experimental studies proving the vector capacity of the species have not yet been performed. Overall, the Chilean trombiculid fauna has only been marginally explored and neither anthropophilic species nor skin reactions caused by chigger bites (trombiculiasis) have so far been described.

Here we report our analyses of the exposure of 3 researchers to chigger mites during a field project in the Aysén Region during the austral summer of 2020, which resulted in trombiculiasis and a subsequent case of scrub typhus.

METHODS

The research team was exposed to chiggers during a field study conducted from 2 to 7 March 2020, in the vicinity of Caleta Tortel, a coastal village in the Aysén Region in southern Chile (47°47′54″S, 73°32′24″W; altitude 360 m above sea level). The 3 researchers collected trombiculid mites and other ectoparasites from trapped rodents in different regions, using a previously described protocol, which did not include the prophylactic use of doxycycline [6]; the results of this study will be presented separately. The study site, located approximately 30 km east of Caleta Tortel, was chosen as the locality of exposure of a previous scrub typhus case (patient number 1 from [3]; Supplementary Figure 1). The research team was in contact with low vegetation while placing and checking the 150 rodent traps. After 48 hours, all team members noticed multiple attached chigger mites, mainly on their lower limbs and inguinal regions (Figure 1A). Subsequently, the sites of the chigger bites transformed into itchy lesions, starting as small papules or wheals and later turning into reddish papules, which lasted for approximately 1 week (Figure 1B).

On day 8 after chigger exposure, 1 of the researchers, a 35-year-old woman, felt progressive fatigue. On the next day, she had low-grade fever (axillary temperature, 38.2°C), headache, photophobia, myalgia, and retroorbital pain. She also noticed a tiny papule with a red halo in her right inguinal region (Figure 1C). Within the next 24 hours, the center of this lesion darkened and later developed into a painless eschar (Figure 1D). Further symptoms included profuse night sweats and a discrete macular exanthema on her trunk and limbs. Empirical treatment with oral doxycycline (100 mg twice daily for 7 days) was initiated, resulting in rapid clinical response within 24 hours. On the following day, blood (ethylenediaminetetraacetic acid [EDTA]) and eschar samples (scab material and swab) were taken and sent to our laboratories in Santiago. DNA was extracted from buffy coat and eschar samples and examined by *Orientia* genus–specific quantitative polymerase chain reaction (qPCR; Orien16S) targeting the 16S rRNA gene (*rrs*) (J Jiang, C Martínez-Valdebenito, T Weitzel, CM Farris, G Acosta-Jamett, K Abarca and AL Richards. Development of a new genus-specific quantitative real-time PCR assay for the diagnosis of scrub typhus in South America, manuscript in preparation),

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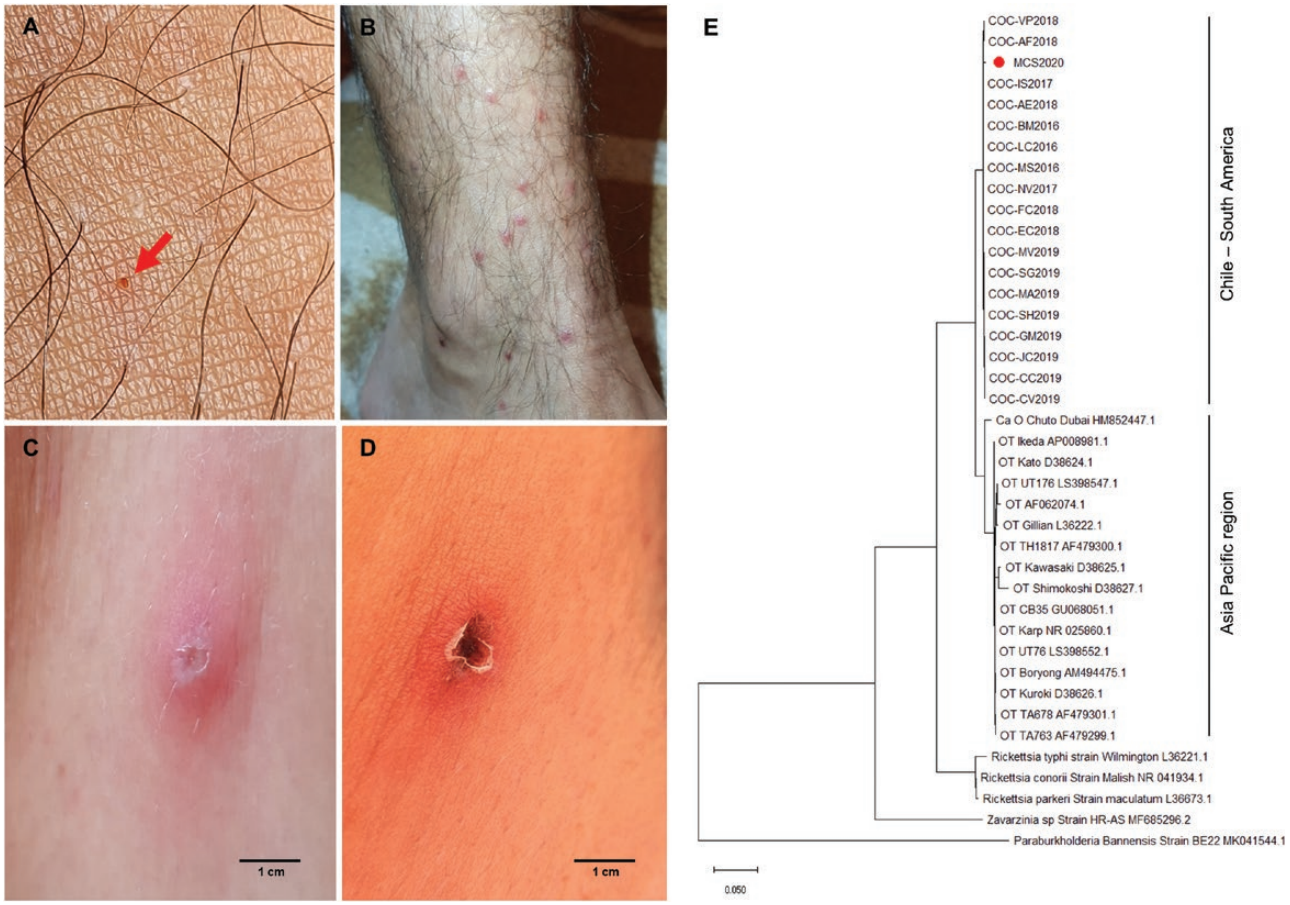

Figure 1. *A*, Chigger mite parasitizing on researcher's skin (arrow). *B*, Trombiculiasis lesions. *C* and *D*, Eschar at days 10 and 12 after chigger bite, respectively. *E*, Phylogenetic analysis of sequences of the 16S rRNA gene (*rrs*) obtained from an eschar sample of the infected researcher (MCS2020, red dot) and from 18 Chilean scrub typhus cases caused by *Candidatus* Orientia chiloensis (COC) [5], as well as sequences from various strains of *Orientia tsutsugamushi* (OT), *Candidatus* Orientia chuto, different *Rickettsia* species, and other microorganisms. We inferred the evolutionary history by using the maximum-likelihood method based on the Kimura 2-parameter model, according to the Bayesian information criterion for these sequences [5]. The analysis involved 40-nt sequences and a total of 900 positions in the final dataset. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. The scale bar indicates nucleotide divergence.

as well as semi-nested PCRs targeting *rrs* and the gene for the 47-kDa high temperature requirement A antigen (*htrA*), as described previously [3].

Trombiculid mite larvae were collected from the 3 field researchers (n = 27) and from the environment by dragging a piece of cloth over low vegetation (n = 23) (Supplementary Figure 1). Samples were collected using a sterile fine-tip forceps and stored in vials with 96% ethanol. All specimens were evaluated by fluorescence microscopy to identify species and feeding status [8]; samples were then tested by Orien16S qPCR [6], except for 10 mites (5 from humans and 5 from the environment), which were cleared in Nesbitt's solution and mounted on slides with Berlese medium [9]. Those specimens were used for further morphological analysis, in cooperation with an expert acarologist in Russia (A. A. S.).

The study was approved by the Comité de Ética en Investigación, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile; affected individuals gave their written consent to publish the presented data.

RESULTS

The clinical diagnosis of scrub typhus was confirmed by molecular testing of eschar samples, which were positive using all 3 molecular assays; buffy coat tested negative. Amplicons obtained from *rrs* semi-nested PCR of eschar samples were sequenced and compared with those from 18 former Chilean patients with scrub typhus and sequences from other *Orientia* and *Rickettsia* species, as previously described [5]. This phylogenetic analysis grouped the strain within the other Chilean isolates, confirming it as *Ca.* O. chiloensis (Figure 1D).

All mite samples obtained from humans and the environment were identified as *Herpetacarus antarctica*. This species was also the only species detected on rodents at the study site (MC Silva-de la Fuente and G Acosta-Jamett, 2021, unpublished observations). Molecular testing using Orien16S qPCR of individual mite samples collected from humans and low vegetation were positive for *Orientia* DNA in 10 of 22 cases (45.4%) and 6 of 18 cases (33.3%), respectively.

DISCUSSION

Although the reported findings were not planned as part of the field project, they provide unique information for our understanding of the transmission of scrub typhus in Chile. First, the sequence of documented chigger bite, trombiculiasis, eschar development at inoculation site, and subsequent fever (scrub typhus), together with the identification of *Orientia* DNA in engorged mites (from patients), unengorged mites (from environment), and patients' eschar samples provided conclusive evidence of the transmission of the Chilean *Orientia* species by *H. antarctica*. This chigger is thus the first directly proven vector of scrub typhus that does not belong to the genus *Leptotrombidium*. Another recently described member of this genus, *H. eloisae*, has been suggested as a scrub typhus vector on Chiloé Island, approximately 650 km north of Caleta Tortel [6]. Second, we observed that *H. antarctica* attacked humans and caused local cutaneous reactions to the mites' saliva secretion, known as trombiculiasis. Interestingly, such skin manifestations are uncommon in trombiculid vectors (*Leptotrombidium* spp.) in Asia-Pacific [10]. In Chile, this clinical entity is probably underdiagnosed and neither commonly known nor systematically studied. Third, our data indicated a very high population density of chiggers, which is in accordance with the high prevalence of chigger infestation of rodents at this location (MC Silva-de la Fuente and G Acosta-Jamett, 2021, unpublished data), suggesting the existence of highly infested hot spots (mite islands). This phenomenon has been described within the tsutsugamushi triangle; in Japan, for example, these localities are known as *yudokuchi* ("poisonous place") [11]. Fourth, the fact that unengorged chiggers collected from the environment were infected with *Orientia* spp. provides proof of vertical transmission, taking into account that chiggers feed on their host only once [12]. This indicated that these mites serve as a reservoir of Chilean *Orientia* species, similar to *O. tsutsugamushi* in Asia-Pacific [11]. Furthermore, the infection rate of 33% suggested a high prevalence of *Orientia* species within the mite population, which, however, requires confirmation by larger surveys. Vector studies of free-living mites from the Asia-Pacific region have demonstrated average infection rates of 2.2%–9.6%, depending on the applied methods [11].

The identified trombiculid species *H. antarctica* (originally described as *Proschoengastia antarctica*) represents the largest and most southward trombiculid species in Chile [13]. It was known only from an American mink (*Neogale vison*) examined on Navarino Island, Magallanes region [13], less than 100 km from a recently described focus of scrub typhus in Tierra del Fuego [4]. In accordance with findings from the northern hemisphere, the chigger fauna in this sub-Antarctic region consisted of only a single species and was less diverse than in higher latitudes in Chile [7, 14]. Importantly, this report demonstrates anthropophilic behavior of *Herpetacarus* species, which has not been described before [15]. This genus is distributed worldwide except for Europe; 9 of its species were described from South America [7].

The study region is located in the Chilean part of Patagonia and belongs to the Magallanic subpolar forests ecosystem, characterized by a complex system of fjords, channels, estuaries, and glaciers. Caleta Tortel is regarded as the most picturesque village in Chile. It is reachable by the Carretera Austral highway and therefore a frequent destination of domestic and international nature tourism, especially during the austral summer months. As indicated by the high attack rate within our research team, travelers in contact with soil or vegetation might be at high risk of acquiring scrub typhus in some parts of this region. Various travel-associated scrub typhus cases have been diagnosed in Chile, raising the question of preventive measures including chemoprophylaxis [4, 16].

In conclusion, our findings demonstrate that trombiculid mites of the species *H. antarctica* are anthropophilic, cause human trombiculiasis, and serve as a vector and reservoir for scrub typhus caused by *Ca.* O. chiloensis in southern Chile.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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