Scrub typhus is a life-threatening zoonosis caused by *Orientia tsutsugamushi* organisms that are transmitted by the larvae of trombiculid mites. Endemic scrub typhus was originally thought to be confined to the so-called “tsutsugamushi triangle” within the Asia–Pacific region. In 2006, however, two individual cases were detected in the Middle East and South America, which suggested that the pathogen was present farther afield. Here, we report three autochthonous cases of scrub typhus caused by *O. tsutsugamushi* acquired on Chiloé Island in southern Chile, which suggests the existence of an endemic focus in South America. (Funded by the Chilean Comisión Nacional de Investigación Científica y Tecnológica and the Wellcome Trust.)

**Summary**

**S**CRUB TYPHUS IS A VECTOR-BORNE ZOONOSIS CAUSED BY *ORIENTIA TSUTSUGAMUSHI*. The infection is transmitted by “chiggers” (i.e., the larvae of trombiculid mites of the genus *leptotrombidium*). Although chiggers feed on various vertebrates, the main reservoir is the vector itself, which maintains *orientia* organisms through transstadial and transovarial transmission. After the bite of an infective chigger, a characteristic necrotic inoculation lesion, termed eschar, can develop, which typically contains high bacterial loads. The microorganism then spreads through the lymphatic fluid and blood, causing systemic manifestations that include fever, rash, and laboratory abnormalities such as elevated levels of C-reactive protein and liver enzymes.

Scrub typhus is endemic in a region called the “tsutsugamushi triangle,” which extends from Pakistan in the west to far eastern Russia in the east to northern Australia in the south. Although the exact burden of disease remains unclear, scrub typhus potentially threatens more than a billion people and is estimated to cause a million clinical cases per year in association with substantial mortality. Scrub typhus is increasingly recognized as a leading cause of treatable febrile illness in regions in which the disease is endemic.

Until 2006, scrub typhus infections had not been confirmed beyond the Asia–Pacific boundaries. In 2006, two independent cases of infection with orientia species were detected in unexpected locations; the first case led to the identification of a new species, *O. chuto*, in the Middle East, and the second case was associated with an orientia-like species of bacteria in southern Chile. Here, we report three scrub typhus cases caused by *O. tsutsugamushi* in Chiloé Island in Chile; their presence suggests that this pathogen may be endemic in this part of South America, which is 12,000 km away from the tsutsugamushi triangle.
All three patients presented at the hospital in Ancud in the northern part of Chiloé Island (Fig. 1A). The patients lived and worked in rural areas in the vicinity and explicitly confirmed that they had never traveled outside of Chile.

**PATIENT 1**

In January 2015, a 38-year-old homemaker was hospitalized with fever and generalized exanthema. Two weeks earlier, she had noticed a transient pruritic rash on her abdomen, which had appeared after she had been collecting firewood in a local forest clearing. The rash disappeared with topical antiallergic treatment, with the exception of a red painless macule below her umbilicus, which grew in size and developed into a necrotic eschar over the course of a week. General symptoms started with abdominal pain 7 days before admission. Three days later, the patient noticed a maculopapular rash on her thorax (Fig. 1B), the appearance of which was followed by a sudden onset of high-grade fever, headaches, and intense myalgia, especially in the calves. Increasing malaise and confusion led to hospitalization; at presentation, she was febrile (body temperature, 40.0°C) and apathetic. A physical examination revealed bilateral conjunctivitis; a generalized maculopapular exanthema involving the trunk, neck, and face; and a black eschar with an erythematous halo on the lower abdomen (Fig. 1C). Laboratory testing revealed an elevated erythrocyte sedimentation rate (49 mm per hour; normal range, 0 to 20) and increased levels of C-reactive protein (120.5 mg per liter), aspartate aminotransferase (336 U per liter), alanine aminotransferase (419 U per liter), and gamma-glutamyltransferase (259 U per liter). The following day, blood samples were obtained and eschar biopsies were performed, and empirical therapy with oral doxycycline was initiated. The fever subsided within 24 hours, and she recovered rapidly without sequelae.

**PATIENT 2**

In January 2016, a 40-year-old construction worker had sudden onset of high fever, chills, night sweats, headaches, myalgia, retro-orbital pain, and photophobia. Two days later, he noticed a generalized maculopapular rash and a necrotic lesion on his right leg (Fig. 1D and 1E). Laboratory testing revealed elevated levels of aspartate aminotransferase (279 U per liter), alanine aminotransferase (419 U per liter), and C-reactive protein (259 U per liter). A tentative diagnosis of soft-tissue infection due to a spider bite was made, and he received cloxacillin and antiinflammatory treatment; however, his condition did not improve. After 2 days, blood and eschar samples were obtained, and therapy was changed to oral doxycycline. The fever subsided within 1 day, and the patient recovered rapidly.

**PATIENT 3**

In February 2016, a 55-year-old farmer presented with a generalized maculopapular rash and necrotic eschar on his upper left thigh that had persisted for 3 weeks (Fig. 1F and 1G). He reported that his initial symptoms were high fever, chills, night sweats, intense headaches, myalgia, and arthralgia that had started 3 weeks earlier, but his fever and systemic symptoms had resolved spontaneously after 1 week. Laboratory testing showed a C-reactive protein level of 114 mg per liter at admission. Scrub typhus was suspected, blood and eschar samples were obtained, and the patient was successfully treated with oral doxycycline.

**METHODS**

**STUDY SITE**

Chiloé Island is the main island of the Chiloé Archipelago and has approximately 155,000 inhabitants; it is accessible to mainland Chile by ferry and flight connections and is an emerging travel destination. Since 2014, the Hospital Ancud (in northern Chiloé) and Hospital Castro (in central Chiloé) have been participating in a survey of possible rickettsial infections in febrile patients. The inclusion criteria are undifferentiated fever accompanied by at least one of the following symptoms or characteristics: generalized rash, eschar, thrombocytopenia, and recent outdoor activities. The survey was approved by Comité de Ética en Investigación, Facultad de Medicina, Pontificia Universidad Católica de Chile, in Santiago, Chile, and by the local health authorities.
Figure 1. Geographic Location and Clinical Presentation of Scrub Typhus Cases in Chile.
Panel A shows a map of Chiloé Island in southern Chile, where all the patients in this case report acquired scrub typhus in rural areas close to the city of Ancud (41°52′14″S, 73°48′58″W). Panels B through G show images of the maculopapular exanthema and eschar of Patient 1 (Panels B and C), Patient 2 (Panels D and E), and Patient 3 (Panels F and G).
Samples

After patients provided written informed consent and agreed to participate in the study, blood samples were collected in Vacutainer serum tubes and EDTA tubes. If an eschar was present, sterile saline-moistened swab specimens, crust specimens, or skin-biopsy specimens were collected in sterile tubes. When possible, a convalescent-phase serum sample was obtained for paired serologic testing. Serum or plasma and buffy-coat samples were separated into aliquots, and all samples were transported within 24 hours at 4°C to Santiago, where they were stored at −20°C before testing. Specimens from Patient 1 were shipped on dry ice to Thailand and Laos for subsequent serologic and molecular analysis.

Table 1. Serologic Results of Serum Samples against Different Orientia tsutsugamushi Antigens. *

<table>
<thead>
<tr>
<th>Assay and Antigen</th>
<th>Cutoff Value</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1 IgM</td>
<td>Day 1 IgG</td>
<td>Day 22 IgM</td>
</tr>
<tr>
<td>InBios ELISA — optical density†</td>
<td>1.08 0.24 2.30 2.51</td>
<td>0.23 0.14 1.03 0.18</td>
<td>0.88 2.06</td>
<td></td>
</tr>
<tr>
<td>Recombinant p56-kD antigens</td>
<td>IgM, 0.37; IgG, 0.41</td>
<td>32 256</td>
<td>&lt;32 128</td>
<td>32 512</td>
</tr>
<tr>
<td>Fuller Laboratories IFA — titer‡</td>
<td>Karp ≥128</td>
<td>32 256</td>
<td>&lt;32 128</td>
<td>32 512</td>
</tr>
<tr>
<td></td>
<td>Kato ≥128</td>
<td>32 128</td>
<td>&lt;32 32</td>
<td>32 2048</td>
</tr>
<tr>
<td></td>
<td>Gilliam ≥128</td>
<td>64 512</td>
<td>&lt;32 64</td>
<td>64 512</td>
</tr>
<tr>
<td></td>
<td>Boryong ≥128</td>
<td>64 512</td>
<td>&lt;32 64</td>
<td>64 512</td>
</tr>
<tr>
<td>ARRL IFA — titer§</td>
<td>Gilliam and Litchfield ≥128</td>
<td>320 160 1280 1280</td>
<td>320 160 1280 1280</td>
<td>320 160 1280 1280</td>
</tr>
<tr>
<td>MORU IFA — titer¶</td>
<td>Karp Dynamic‖</td>
<td>640 640 640 1280</td>
<td>640 640 640 1280</td>
<td>640 640 640 1280</td>
</tr>
<tr>
<td></td>
<td>Kato Dynamic‖</td>
<td>640 640 640 1280</td>
<td>640 640 640 1280</td>
<td>640 640 640 1280</td>
</tr>
<tr>
<td></td>
<td>Gilliam Dynamic‖</td>
<td>640 640 640 1280</td>
<td>640 640 640 1280</td>
<td>640 640 640 1280</td>
</tr>
<tr>
<td></td>
<td>Sido Dynamic‖</td>
<td>640 640 640 1280</td>
<td>640 640 640 1280</td>
<td>640 640 640 1280</td>
</tr>
</tbody>
</table>

* Day numbers refer to time after the onset of fever. A dash indicates that the test was not performed.
† The InBios IgM and IgG enzyme-linked immunosorbent assay (ELISA) (Scrub Typhus Detect, InBios International) is based on recombinant 56-kD type-specific antigens of the Karp, Kato, Gilliam, and TA716 strains; the cutoff optical densities indicating positivity are those recommended by the manufacturer.
‡ The Fuller Laboratories IgG indirect immunofluorescent antibody assay (IFA) used four distinct acetone-fixed O. tsutsugamushi strains (Gilliam, Karp, Kato, and Boryong).
§ The Australian Rickettsial Reference Laboratory (ARRL) commercial IgM and IgG IFA used a mixed antigen containing the Gilliam and Litchfield strains.
¶ The Mahidol–Oxford Tropical Medicine Research Unit (MORU) in-house IgM and IgG IFA was based on four separate O. tsutsugamushi strains (Gilliam, Karp, Kato, and Sido).
‖ In the absence of a regional positivity cutoff titer, a dynamic increase in titer to a value 4 times as high was considered to indicate positivity.

SEROLOGIC TESTING

Indirect immunofluorescent antibody assays (IFAs) and enzyme-linked immunosorbent assays (ELISAs) were performed to determine the serologic status of the patients. Initially, all samples were analyzed in Chile with the use of commercial IFA and ELISA kits (Table 1) in accordance with the manufacturers’ instructions. The serologic test results for Patient 1 were confirmed at the Mahidol–Oxford Tropical Medicine Research Unit in Thailand (Table 1). The IFA end-point titers were designated as the highest reciprocal titer showing positivity, and seroconversion was defined as an IgM or IgG antibody titer in the convalescent-phase serum sample that was at least 4 times as high as the titer in
the acute-phase serum sample. The ELISA positivity cutoff values were chosen in accordance with the manufacturer’s instructions, because no predefined regional cutoff values exist. To assess for cross-reactivity, serum samples were tested for IgG antibodies against other members of the order Rickettsiales (Table S1 in the Supplementary Appendix). Molecular analysis of samples from Patient 1 was performed at Lao-Oxford-Mahosot Hospital–Wellcome Trust Research Unit (LOMWRU) in Laos. Nested polymerase-chain-reaction (PCR) assays for gene-specific primers for the full-length genes encoding the 47-kD HtrA protein and 56-kD type-specific antigen (hereafter referred to as the 47-kD and 56-kD genes) were performed with eschar and buffy-coat DNA as described in Figure 2. PCR amplicons were sequenced by Macrogen (Seoul, South Korea), and a 286-bp sequence of the 56-kD gene was used for phylogenetic reconstruction, which included 28 sequences of orientia species selected from GenBank, aligned with the use of ClustalW (www.clustal.org) and compared by the maximum-likelihood method. Specimens from Patients 2 and 3 were tested for the 56-kD gene in Chile in accordance with LOMWRU protocols (Fig. 2). In addition, all buffy-coat and eschar samples were analyzed with genus-specific PCR protocols for the detection of rickettsia, ehrlichia, and anaplasma (see the Supplementary Appendix).

RESULTS

SEROLOGIC TESTING
Anti-O. tsutsugamushi IgM and IgG positivity in Patient 1 was confirmed by two independent laboratories. IgG seroconversion was documented with the use of five distinct antigens; the highest titers were observed with antigens from the Karp strain, Gilliam strain, and a combination of the Gilliam and Litchfield strains, and the highest titer increases from the first day after onset of fever to day 22 were observed with antigens from the Gilliam strain, Sido strain, and a combination of the Gilliam and Litchfield strains (Table 1). IgM and IgG positivity in Patients 2 and 3 was confirmed with the use of commercial ELISA and IFA kits (Table 1). Serologic tests for other organisms in the order Rickettsiales were negative in acute-phase samples and convalescent-phase samples from all patients (Table S1 in the Supplementary Appendix).

MOLECULAR AND PHYLOGENETIC ANALYSIS
Molecular targets specific for orientia species (the 56-kD gene and 47-kD gene) were amplified from the eschar material and buffy coat of Patient 1, which confirmed the presence of orientia DNA (Fig. 2). The 56-kD PCR product of the eschar specimen was sequenced, and Basic Local Alignment Search Tool (BLAST) analysis
A Agarose-Gel Electrophoresis of PCR products

B Phylogenetic Analysis

<table>
<thead>
<tr>
<th>BC samples</th>
<th>Eschar-1</th>
<th>Eschar-2</th>
<th>Positive control</th>
<th>Negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>

47 kD

56 kD

1000 bp

300 bp

AB617619.1, Karp, Japan
AB173043.1, Karp, Japan
AB617606.1, Karp, Japan
AF173045.1, Okazaki, Japan
AF173046.1, Kamimoto, Japan
AF173044.1, Mori, Japan
M33004.1, Karp, Korea
HM77470.1, PG9, Thailand
L04956.1, Boryong, Korea
M63380.1, Kuroki, Japan
DQ485289.1, Gilliam, Taiwan
AF173036.1, Ikeda, Japan
AF173035.1, Iwataki, Japan
AY243357.1, Hualien, Taiwan
KC456654.1, JN2, China
KC456653.1, YN9-09, China
AF173038.1, Taguchi, Japan
M63383.1, Kawasaki, Japan
U19904.1, TA678, Thailand
U19905.1, TA716, Thailand
KU756137, Chiloe, Chile 2015
AB715376.1, Kato, Japan
AF173041.1, Akita, Japan
AF173040.1, Omagari, Japan
AF173040.1, Omagari, Japan
M63382.1, Kato, Japan
M63381.1, Shimokoshi, Japan
HM852448.1, Chuto, Dubai
revealed a close relationship with *O. tsutsugamushi*, with homologies ranging from 94 to 97%. Subsequent phylogenetic analysis grouped the sequence with the Kato-related strains (Fig. 2). The sequence was deposited in GenBank (accession number, KU756137). For Patient 2, the 56-kD gene was amplified from eschar material. Molecular tests of samples from Patient 3 remained negative, probably because the samples were collected late (14 days after the resolution of fever). Results of PCR assays for the detection of rickettsia, ehrlichia, and anaplasma DNA remained negative (Table S1 in the Supplementary Appendix).

**DIAGNOSIS OF *O. TSUTSUGAMUSHI* INFECTION**

Patient 1 had seroconversion detected in 7 of 14 IFAs, had positive IgM and IgG ELISA results, and had positive PCR results for the 56-kD and 47-kD genes in blood and eschar samples; sequencing of the 56-kD amplicon (from eschar) revealed a high degree of homology with *O. tsutsugamushi*. Patient 2 had seroconversion detected in 2 of 4 IFAs and had positive IgM ELISA and 56-kD PCR (eschar) results. Patient 3 had two high single-titer IFA results. No evidence for infection with other rickettsial organisms or Anaplasmataceae was found in serologic or molecular investigations.

**DISCUSSION**

Scrub typhus is a neglected bacterial zoonosis and an underdiagnosed and underreported febrile illness that leads to hospitalization in regions in which the disease is endemic. Endemic scrub typhus has almost exclusively been reported from within the tsutsugamushi triangle. This case series from southern Chile shows that scrub typhus due to *O. tsutsugamushi* occurs in the Western hemisphere.

The origin of the South American *O. tsutsugamushi* strain remains to be investigated. *O. tsutsugamushi* is made up of more than 20 known antigenically distinct genotypes, which differ in their geographic distribution and in the clinical presentation caused by infection. Although genotyping of orientia has focused primarily on the type-specific 56-kD gene, which places this Chilean isolate within the geographically diverse Kato-related clade, more in-depth and preferably whole-genome sequencing efforts could reveal associations with related strains from other geographic areas. Similar to other rickettsioses, scrub typhus is maintained within an ecosystem as a result of complex interactions among the environment, vertebrates, arthropod vectors, and the pathogens. Hotspots of scrub typhus (often termed “mite islands”) are associated with secondary vegetation and an abundance of trombiculid mites. Indeed, large parts of Chiloé Island consist of secondary vegetation, and only fragments of the original Valdivian temperate rainforest are left. Chiloé has an oceanic climate with cool, wet winters and mild, dry summers; although the number of patients is still too small to draw conclusions with regard to seasonality, the appearance of all cases of scrub typhus during January and February suggests that summer may be the main activity period of the yet-unknown vector.

The Chilean patient whose condition was diagnosed in 2006 had several leech bites, and the authors of the case report speculated about a possible leech-transmitted orientia lineage. Rickettsial organisms have been detected in these ectoparasites, and discussions about leech-borne rickettsial infections have been revived by a report from Laos. Our patients reported that they had not been bitten by leeches; however, all the patients reported the handling or collecting of firewood, which has previously been identified as a risk factor for chigger bites in Asia. In addition, Patient 1 had pruritic lesions compatible with chigger bites (trombiculiasis) before the onset of febrile illness.

Scrub typhus is a potentially serious and easily treatable infection, but common first-line antibiotics — such as beta-lactams — that are used for the treatment of undifferentiated febrile illnesses are ineffective. An increase in the general awareness of scrub typhus is required to improve the management of illness in febrile patients living in or returning from regions in which the disease is endemic. This report highlights the fact that our understanding of the global epidemiologic profile of scrub typhus is limited. The development of accurate and affordable diagnostic tools for rickettsial illnesses is a major need. New molecular methods have contributed substantially to the identification and characterization of rickettsial infections around the world, but they are often not available in regions in which these infections are endemic. Routine diagnosis still requires serologic detection with the use of a suboptimal IFA, but we hope that
recent advances such as scrub typhus rapid diagnostic tests and ELISAs with improved diagnostic accuracy will contribute toward broadening detection. Nevertheless, serologic cross-reactivity with other rickettsiae is possible, and in regions such as South America, where old and new rickettsia species have emerged in recent years, a thorough diagnostic workup is required to distinguish scrub typhus from other rickettsial infections that cause rash and eschar.

This investigation showed the presence of scrub typhus in southern Chile. Although the distribution, reservoirs, and vectors remain unknown, this finding expands our understanding of the epidemiology of scrub typhus and suggests that there may be a much wider global distribution and higher disease burden than has previously been appreciated.

Supported by grants from the Comisión Nacional de Investigación Científica y Tecnológica (Fondo Nacional de Desarrollo Científico y Tecnológico grant 1130817 to Des. Weitza, López, and Abacar) and the Wellcome Trust (to the Mahidol–Oxford Tropical Medicine Research Unit and the Lao-Oxford-Mahoset Hospital–Wellcome Trust Research Unit).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Marina Pereira and Andrés Hernández for valuable clinical information and documentation of the cases in the Hospital Ancud, Teresa Azócar for technical assistance, Gerardo Acosta-Jamett for his help with the graphic material, and Paul Newton (Lao-Oxford-Mahoset Hospital–Wellcome Trust Research Unit) for his useful comments during the preparation of an earlier version of the manuscript.

REFERENCES


Copyright © 2016 Massachusetts Medical Society.

AN NEJM APP FOR iPHONE

The NEJM Image Challenge app brings a popular online feature to the smartphone. Optimized for viewing on the iPhone and iPod Touch, the Image Challenge app lets you test your diagnostic skills anytime, anywhere. The Image Challenge app randomly selects from 300 challenging clinical photos published in NEJM, with a new image added each week. View an image, choose your answer, get immediate feedback, and see how others answered. The Image Challenge app is available at the iTunes App Store.