BRIEF REPORT

Endemic Scrub Typhus in South America

Thomas Weitzel, M.D., Sabine Dittrich, Ph.D., Javier López, D.V.M., Weerawat Phuklia, M.Sc., Constanza Martinez-Valdebenito, B.Sc., Katia Velásquez, M.D., Stuart D. Blacksell, Ph.D., Daniel H. Paris, M.D., and Katia Abarca, M.D.

SUMMARY

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.)

From Clínica Alemana de Santiago, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo (T.W.), Veterinary Hospital Puente Alto (J.L.), and Departamento de Enfermedades Infecciosas e Inmunología Pediátrica (C.M.-V., K.A.) and Millennium Institute on Immunology and Immunotherapy (K.A.), Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, and Hospital de Ancud, Ancud (K.V.) — all in Chile; Lao-Oxford-Mahosot Hospital–Wellcome Trust Research Unit, Vientiane, Laos (S.D., W.P.); Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom (S.D., S.D.B., D.H.P.); and Mahidol–Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand (S.D.B., D.H.P.). Address reprint requests to Dr. Abarca at the Infectious Diseases and Molecular Virology Laboratory, Pontificia Universidad Católica de Chile, Marcoleta 391, Santiago, Chile, or at katia@med.puc.cl; or to Dr. Weitzel at the Laboratorio Clínico, Clínica Alemana de Santiago, Vitacura 5951, Santiago, Chile, or at tweitzel@alemana.cl.

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Scrub 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.[1] 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.[2]

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.[3] Scrub typhus is increasingly recognized as a leading cause of treatable febrile illness in regions in which the disease is endemic.[1,4,5]

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,[6] and the second case was associated with an orientia-like species of bacteria in southern Chile.[7] 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.

CASE REPORTS

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; normal range, 0 to 5.0), aspartate aminotransferase (279 U per liter; normal range, 0 to 35), alanine aminotransferase (419 U per liter; normal range, 10 to 50), and gamma-glutamyltransferase (259 U per liter; normal range, 0 to 35). 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 (336 U per liter), alanine aminotransferase (395 U per liter), and C-reactive protein (49.9 mg 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.

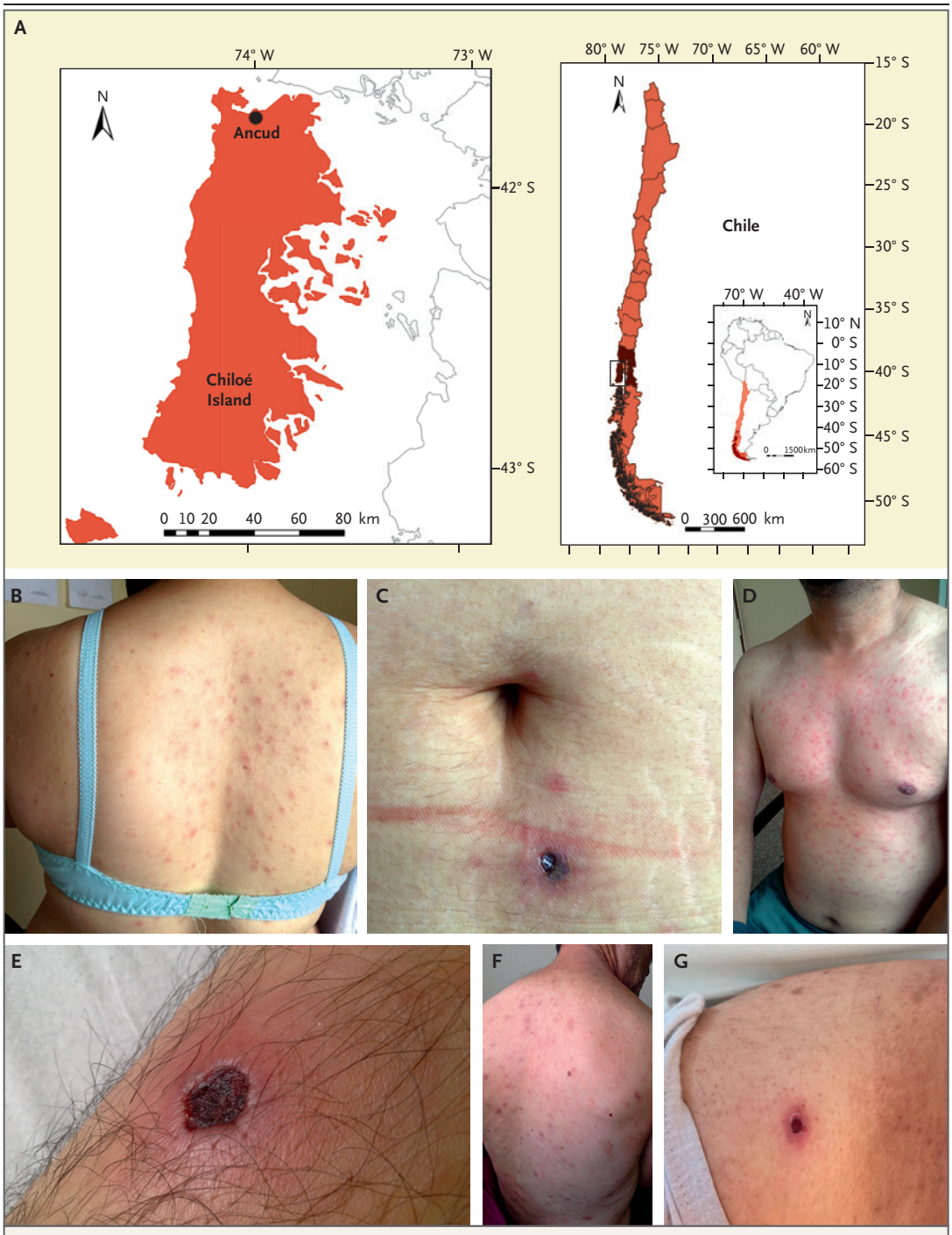


Figure 1. Geographic Location and Clinical Presentation of Scrub Typhus Cases in Chile.

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).

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

Assay and Antigen	Cutoff Value	Patient 1				Patient 2				Patient 3	
		Day 1 IgM	Day 1 IgG	Day 22 IgM	Day 22 IgG	Day 6 IgM	Day 6 IgG	Day 34 IgM	Day 34 IgG	Day 21 IgM	Day 21 IgG
InBios ELISA — optical density†											
Recombinant p56-kD antigens	IgM, 0.37; IgG, 0.41	1.08	0.24	2.30	2.51	0.23	0.14	1.03	0.18	0.88	2.06
Fuller Laboratories IFA — titer‡											
Karp	≥128	—	32	—	256	—	<32	—	128	—	512
Kato	≥128	—	32	—	128	—	<32	—	32	—	2048
Gilliam	≥128	—	64	—	512	—	<32	—	64	—	512
Boryong	≥128	—	32	—	64	—	<32	—	128	—	1024
ARRL IFA — titer§											
Gilliam and Litchfield	≥128	320	160	1280	1280	—	—	—	—	—	—
MORU IFA — titer¶											
Karp	Dynamic‖	640	640	640	1280	—	—	—	—	—	—
Kato	Dynamic‖	320	160	640	640	—	—	—	—	—	—
Gilliam	Dynamic‖	640	320	1280	640	—	—	—	—	—	—
Sido	Dynamic‖	160	160	640	320	—	—	—	—	—	—

* 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,[8] a dynamic increase in titer to a value 4 times as high was considered to indicate positivity.

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.

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.[9] 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, available with the full text of this article at NEJM.org).

MOLECULAR ANALYSIS

The 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.[6] 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).

Figure 2 (facing page). Molecular Confirmation of *Orientia tsutsugamushi* Infection

Panel A shows the polymerase-chain-reaction (PCR) products after agarose-gel electrophoresis for the 56-kD gene (product size, 620 bp) and 47-kD gene (product size, 1401 bp) amplification obtained from buffy-coat preparations (BC samples) and eschar-biopsy specimens (eschar-1, unfixed; eschar-2, formalin-fixed) with the use of extended cycling conditions. All samples were run in duplicate together with an isolate of *O. tsutsugamushi* from a patient from Laos (positive control, lanes 7 and 8 and lanes 17 and 18 [the product size was distorted as a result of sample overloading]) and negative controls (lanes 9 and 10 and lanes 19 and 20) as external controls. The controls repeatedly and reliably showed the correct result. For the 47-kD PCR assay, lanes 1 and 2 show the correct amplicons at 1000 bp for buffy-coat samples. In lanes 3 and 4, the eschar-1 specimen shows multiple weak bands at the target size, and the eschar-2 (lanes 5 and 6) is negative. For the 56-kD PCR assay, lanes 11 and 12 show no amplification of the correct size from the buffy-coat sample. The eschar-1 and eschar-2 samples show the correct size amplicon in three of four reactions. PCR products were visualized on a 1.5% agarose gel before purification with a QIAquick gel extraction kit (Qiagen) in accordance with the manufacturer's instructions. Panel B shows a phylogenetic analysis of the 286-bp sequence of the 56-kD gene obtained with the pooled eschar amplicons from Patient 1 (KU756137, Chiloe, Chile 2015). The evolutionary history was inferred with the use of the maximum-likelihood method based on the Tamura–Nei model. The tree with the highest log likelihood (–1479.2759) is shown. Bootstrap values are shown next to the branches. We obtained initial trees automatically by applying the Neighbor-Join and BioNJ algorithms in Molecular Evolutionary Genetics Analysis software, version 6.0 (MEGA6), to a matrix of pairwise distances estimated with the maximum composite likelihood approach and then selecting the topology with superior log-likelihood value. The analysis involved 29 nucleotide sequences.

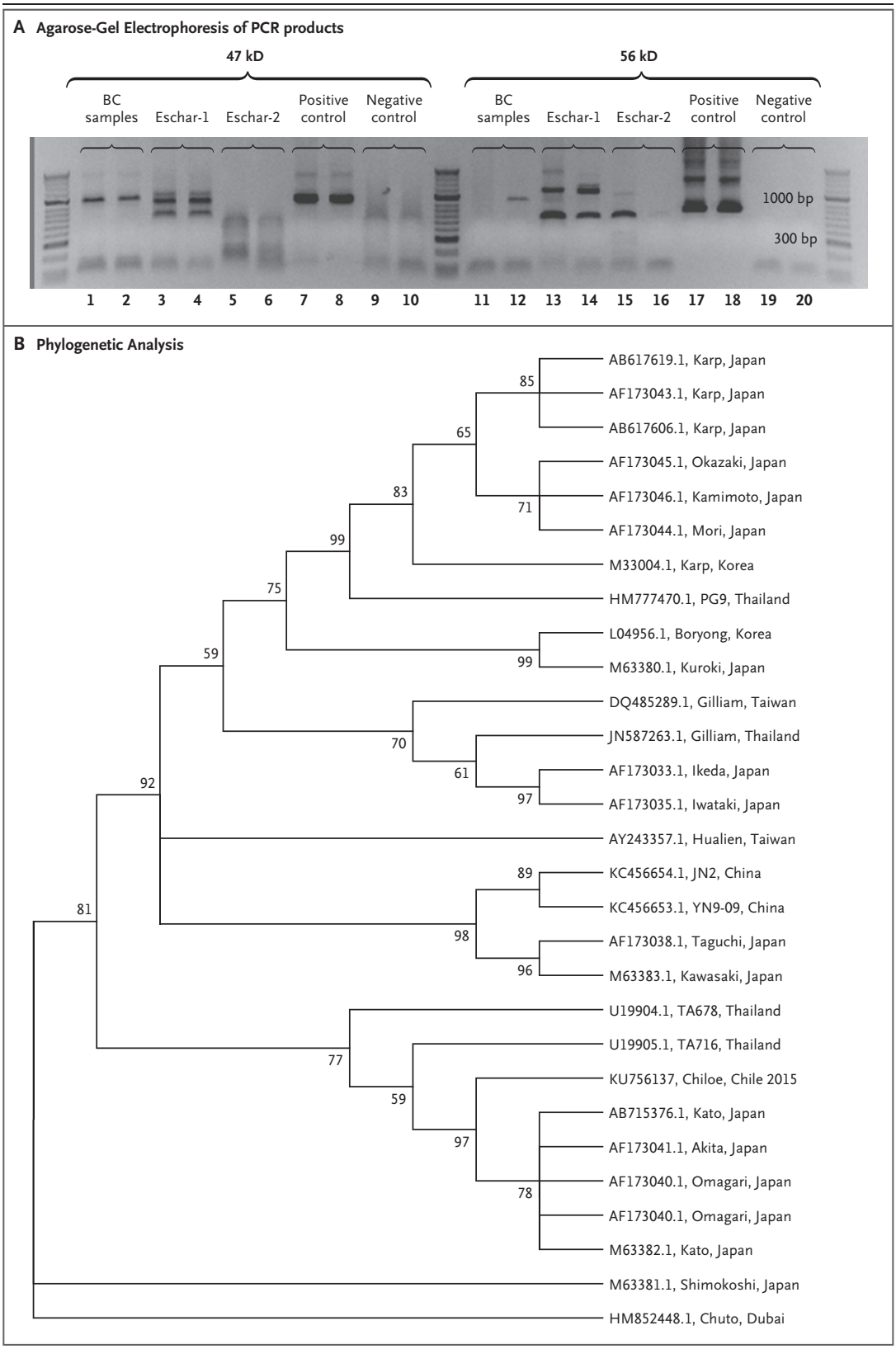
RESULTS

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)[6,10,11] 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
47 kD
56 kD
BC samples
Eschar-1
Eschar-2
Positive control
Negative control
BC samples
Eschar-1
Eschar-2
Positive control
Negative control
1000 bp
300 bp
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
B Phylogenetic Analysis
AB617619.1, Karp, Japan
AF173043.1, Karp, Japan
AB617606.1, Karp, Japan
AF173045.1, Okazaki, Japan
AF173046.1, Kamimoto, Japan
AF173044.1, Mori, Japan
M33004.1, Karp, Korea
HM777470.1, PG9, Thailand
L04956.1, Boryong, Korea
M63380.1, Kuroki, Japan
DQ485289.1, Gilliam, Taiwan
JN587263.1, Gilliam, Thailand
AF173033.1, 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
85
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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.[12,13] 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.[3] 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.[14] Hotspots of scrub typhus (often termed "mite islands") are associated with secondary vegetation and an abundance of trombiculid mites.[14] Indeed, large parts of Chiloé Island consist of secondary vegetation, and only fragments of the original Valdivian temperate rainforest are left.[15] 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.[7] Rickettsial organisms have been detected in these ectoparasites, and discussions about leech-borne rickettsial infections have been revived by a report from Laos.[16,17] 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.[18] 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.[4] 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.[19] New molecular methods have contributed substantially to the identification and characterization of rickettsial infections around the world,[20] 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,[19] but we hope that

recent advances such as scrub typhus rapid diagnostic tests and ELISAs with improved diagnostic accuracy will contribute toward broadening detection.[9] Nevertheless, serologic cross-reactivity with other rickettsiae is possible,[21] and in regions such as South America, where old and new rickettsia species have emerged in recent years,[22,23] 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 then has previously been appreciated.

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

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