sample, but the MICs of bedaquiline, clofazimine, and delamanid were above the breakpoints typically considered to be resistant (*4*). In the month 42 sputum sample, WGS found resistance mutations to bedaquiline and clofazimine, consistent with the MIC testing, but there were multiple resistance mutations to delamanid, even though the MIC of delamanid was below the typical breakpoint for resistance. WGS and MIC testing were consistent for fluoroquinolones (resistant) and linezolid (susceptible) in sputum samples from both months.

Complicating interpretation of WGS is the fact that many resistance mutations for the new and repurposed drugs have not yet been discovered. In the patient we treated, who was symptomatic and bacteriologically sputum positive for many months on a regimen containing bedaquiline, clofazimine, delamanid, and linezolid, the mutation conferring resistance to fluoroquinolones, Asp94Ala, is well known, but none of the other mutations found (Figure, panel C) have been previously reported in the scientific literature as conferring resistance to bedaquiline/clofazimine (Rv0678_Arg38Leu, Rv0678_Arg123Met) or delamanid (ddn_Pro96His, fbiA_Arg234Leu, fbiB_Pro361His, fbiB_Gly422Val) (*5*). We considered those to be true resistance mutations because they are located in relevant genes, and the clinical, bacteriologic, and radiologic evidence is consistent with resistance acquisition.

Even with new drugs and regimens, treating MDR TB will continue to be challenging. As both phenotypic and genotypic resistance testing for new and repurposed TB drugs continues to evolve, so must our understanding of how resistance testing correlates with clinical response.

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Orientia tsutsugamushi Antibodies in Patients with Eschars and Suspected Tickborne Disease

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To investigate local transmission of *Orientia tsutsugamushi* by chiggers in North Carolina, USA, we tested remnant serum specimens from patients with eschar undergoing testing for suspected tickborne disease. We identified 11 persons with *O. tsutsugamushi* antibodies, including 4 who were positive by both assays; none had severe clinical manifestations consistent with scrub typhus.

[1]These first authors contributed equally to this article.

Scrub typhus, caused by *Orientia tsutsugamushi* bacteria and transmitted by Trombiculid mites, affects >1 million persons each year (*1*,*2*). Clinical disease is characterized by fever, headache, and eschars. Untreated, case-fatality rates for the disease can exceed 30% (*2*,*3*).

Most cases of scrub typhus occur in Asia and the Pacific. Although the vectors, commonly known as chiggers, are widely distributed across North America, no autochthonous (i.e., locally acquired) cases have been reported in the United States. In 2023, however, researchers identified *O. tsutsugamushi* in chiggers collected in North Carolina (*4*). To investigate potential transmission to humans in North Carolina, we sought to estimate the seroprevalence of *O. tsutsuguamushi* antibodies and assess the clinical characteristics of persons with evidence of exposure. We hypothesized that cases of eschar are more likely to be caused by endemic tickborne Rickettsiaceae, such as *Rickettsia parkeri* bacteria (*5*).

The protocols of the parent study have been published (*6*). In brief, we collected remanent serum specimens from adult patients undergoing testing for spotted fever group *Rickettsia* or *Ehrlichia* spp. bacteria as part of routine care for acute febrile illness. For the purposes of this study, we selected serum specimens from patients with documented eschar.

We performed serologic testing in parallel by using 2 commercially available kits. We performed all tests according to manufacturers' instructions and ran the tests with the included positive and negative controls. We first tested samples by using IgM-specific indirect immunofluorescence antibody (IFA) assays against *O. tsutsugamushi* (Fuller Laboratories, https://fullerlaboratories.com) (*7*). We diluted samples to a reciprocal titer of 1:64. In parallel, specimens underwent testing with the Scrub Typhus Detect ELISA-based assays that detect IgM and IgG (InBios International Inc., https://inbios.com). We diluted samples to a reciprocal titer of 1:100. The IgG ELISA kit instructions suggested an optical density (OD) cutoff of 0.37, but the IgM kit did not suggest a cutoff. We applied the 0.37 OD cutoff to the IgG results but also applied several IgM and IgG OD cutoffs from existing literature (*8*,*9*) (Table 1). We estimated seropositivity and used the Clopper-Pearson exact method to calculate 95% CIs.

A total of 138 (5.3%) of 2,593 persons had an eschar documented. Of those, 101 had an adequate sample volume. On the basis of the number of slides and kits available, we selected 87 (86.1%) samples for IFA and 92 (91.1%) samples for ELISA from 83 unique person; some persons had both acute and convalescent samples tested. Of the 83 persons, 35 (42.1%) had illnesses that had been classified as confirmed, probable, or suspected cases of spotted fever group *Rickettsia* (*10*) (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/31/11/25-0763-App.pdf).

By IFA result, we classified 18 (20.6%) of 87 samples as indeterminate (i.e., weakly positive). By ELISA result, 4 (4.3%) of 92 were positive for *O. tsutsugamushi* IgM and 8 (8.9%) of 90 for IgG; no samples were positive for both. None of the 18 samples that were indeterminate by IFA were positive by ELISA. The 8 samples IgG-positive by ELISA results underwent confirmatory IgG IFA testing at Fuller Laboratories (Appendix Table 2). We observed 50% (4 of 8) agreement in IgG reactivity between ELISA and IFA results at an endpoint titer of 1:128.

Of the 11 persons with either a reactive IgG or IgM, 7 (63.6%) reported a tick bite. None of the clinical encounters were associated with travel. Fever and myalgia were the most common clinical syndrome (Table 2). All patients were seen in outpatient settings, and none were hospitalized.

Our study identified North Carolina residents with antibodies reactive against *O. tsutsugamushi* bacteria. However, those results must be interpreted cautiously. The presence of antibodies does not indicate active infection but can be a marker of prior exposure. In addition, in a setting where transmission has not been previously reported, positive results are more likely to be falsely positive because of imperfect test performance. However, the presence of antibodies, especially when indicated by both assays, was unexpected and merits investigation.

Including only persons who had reactive antibodies by both assays (n = 4), our estimate of IgG

Table 1. Seropositivity estimates based on IgM and IgG ELISA at varying optical density thresholds in study of *Orientia tsutsugamushi* bacteria in patients with eschars and suspected tickborne disease, North Carolina, USA, 2020–2022*

Test	Optical density cutoff	Sensitivity	Specificity	Positive, n = 92	% Seropositive (95% CI)
IgG ELISA	0.37	NR	NR	8	8.7 (3.8–16.4)
IgG ELISA (*8*)	1.0	97.5	60	1	1.1 (0.0–5.9)
IgG ELISA (*8*)	1.6	91	75	0	0.0 (0.0–3.9)
IgM ELISA (*8*,*9*)	0.6	96.4	82.7	2	2.2 (0.3–7.6)
IgM ELISA (*8*)	0.492	97.1	79.1	4	4.3 (1.2–10.8)

*NR, not reported.

Table 2. Demographic, clinical, and laboratory test results of persons with antibodies against *Orientia tsutsugamushi* in study of *O. tsutsugamushi* among patients with eschars and suspected tickborne disease, North Carolina, USA, 2020–2022*

Person no.	Age, y/sex	IFA result	ELISA result	Tick bite	Fever	Headache	Myalgia	Rash	SFGR classification	*Ehrlichia* classification
1	42/M	IgG+	IgG+	Yes		X		X	Probable	Probable
2	48/M	IgG+	IgG+	Yes	X	X	X		NAC	Unknown
3	66/M	IgG+	IgG+	Yes		X	X	X	NAC	Probable
4	55/F	IgG+	IgG+	Unknown	X	X			NAC	NAC
5	76/F	Negative	IgG+	Yes					Probable	Probable
6	64/M	Negative	IgG+	Yes	X		X		Probable	NAC
7	43/F	Negative	IgG+	Unknown					NAC	Unknown
8	60/F	Unknown	IgM+	Yes			X		NAC	Probable
9	44/M	Negative	IgM+	Unknown					Probable	Unknown
10	78/F	Unknown	IgM+	No	X				Unknown	NAC
11	72/F	Unknown	IgM+	Yes	X		X		Probable	Probable

*IFA, immunofluorescence antibody; NAC, not a case (i.e., did not meet Council of State and Territorial Epidemiologists criteria); SFGR, spotted fever group *Rickettsia*; Unk, unknown or not reported; X, sign or symptom present in patient.

seroprevalence is 4.3% (95% CI 1.2%–10.8%) but, depending on the assay and cutoffs applied, might be as high as 8.9. However, if *O. tsutsugamushi* bacteria were being locally transmitted, we would expect cases of severe illness, which we did not observe.

One limitation of this study is its reliance on serologic results and absence of contextual information, including travel histories and clinical outcomes. In addition, most patients did not have convalescent titers drawn, so we were unable to confirm cases.

We believe the current evidence does not support transmission of virulent *O. tsutsugamushi* strains in North Carolina. Further investigation, ideally by using molecular approaches from clinical samples (e.g., eschar swab samples), is needed. Clinicians should maintain a high level of suspicion for *R. parkeri* rickettsiosis, the seroprevalence of which was nearly 10 times greater than *O. tsutsugamushi*, when evaluating patients with arthropod-associated eschars.

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Study activities for the parent study were reviewed by the UNC Institutional Review Board (21-0356). Some activities, including the collection and testing of remnant samples, were granted a waiver of informed consent.

Deidentified individual data that supports the results will be shared after publication provided the investigator who proposes to use the data has approval from an institutional review board, independent ethics committee, or research ethics board, as applicable, and executes a data use and sharing agreement with University of North Carolina.

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Mortality Event in Rainbow Snakes Linked to Snake Fungal Disease, United States

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We report mortality in rainbow snakes in Virginia and North Carolina, USA, linked to snake fungal disease caused by *Ophidiomyces ophidiicola*. During 2013–2023, we observed 46 dead rainbow snakes with lesions indicative of snake fungal disease, noted elevated disease severity compared with other species, and recorded fewer live snakes over time.

Detecting and assessing declines in elusive or rare species can be difficult. Early identification of populations in decline can help accelerate intervention strategies and reduce the likelihood of genetic bottlenecks, population extirpation, and trophic disturbances of ecologically important species (*1*). Snake fungal disease (SFD) is caused by the fungal pathogen *Ophidiomyces ophidiicola* and affects a broad range of snake species (*2*), causing skin lesions as the fungus invades tissues, sometimes leading to impaired movement, anorexia, and even death (*3*). Researchers have documented population impacts from SFD in 2 snake species (*4*,*5*), but the extent of mortality across snake species is likely underestimated due to the cryptic nature of snakes. We describe a multiyear mortality event associated with SFD in a rare species, the rainbow snake (*Farancia erytrogramma*), in Virginia and North Carolina, USA.

In spring 2019, regional biologists from the Back Bay region of North Carolina and Virginia reported 6 deceased rainbow snakes. In spring of 2020, we found an additional 6 snakes in the same area (Figure 1, panel A). After those events, we gathered additional records of dead rainbow snakes (Appendix Table 1, https://wwwnc.cdc.gov/EID

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