Case Report: Scrub Typhus and Q Fever Coinfection

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Abstract. A 56-year-old female goat herder had scrub typhus that persisted after receiving doxycycline for 5 days. Her symptoms continued, prompting us to perform further examinations that revealed coinfection of Q fever and scrub typhus via molecular and serological testing. We also isolated *Orientia tsutsugamushi* using BALB/c mice and L929 cells.

Q fever is a zoonotic infection caused by *Coxiella burnetii*. Although mammals, birds, and arthropods are all reservoirs of *C. burnetii*, ticks are known to be one of the main carriers.¹ The most common source of human infections is farm animals, including cattle, goats, and sheep.²

Scrub typhus is a mite-borne infectious disease caused by *Orientia tsutsugamushi*. The disease occurs predominantly in Asian Pacific Rim countries, including South Korea, China, Taiwan, Japan, India, Thailand, and Australia.³

To date, almost no study has reported coinfection of Q fever and scrub typhus.⁴ Here, the patient's scrub typhus persisted even after she received doxycycline for 5 days. Her continuing symptoms prompted us to perform further examinations, in which we identified coinfection of Q fever and scrub typhus molecularly and serologically. In addition, we isolated *O. tsutsugamushi*. This report describes the details of the case.

THE STUDY

A 56-year-old woman had symptoms of fever, headache, nausea, and vomiting for 1 week before visiting our hospital (October 25, 2016). She eventually presented to the emergency department (ED) of Chosun University Hospital (November 1, 2016). The ED physician detected rashes on her body and an eschar in the abdomen. A complete blood count (CBC) test yielded the following findings: hemoglobin level, 15.4 g/dL; white blood cell (WBC) count, 10,270/mm³; platelet count, 109,000/mm³; aspartate aminotransferase/alanine aminotransferase (AST/ALT), 100.7/199.8 U/L; and C-reactive protein level, 14.59 mg/dL. The doctor at the ED suspected scrub typhus and prescribed doxycycline for 5 days.

After 5 days of oral doxycycline treatment, she continued to experience myalgia, which led her to visit our ED again (November 7, 2016). The ED doctor performed laboratory tests again, and the CBC test findings were as follows: hemoglobin level, 13.6 g/dL; WBC, 10,190/mm³; platelet count, 397,000/mm³; and AST/ALT, 134.9/109.6 U/L. The doctor at the ED thought that the patient had post-scrub typhus asthenia; briefly, the doctor determined that the scrub typhus had been cured but that general weakness had continued. Therefore, the doctor did not prescribe further antibiotics. Instead,

analgesics were prescribed. She was discharged. A week later, however, she visited the outpatient clinic of the Division of Infectious Diseases (November 14, 2017), complaining of severe fatigue that prevented her from walking. The patient was a goat herder. Her goats never miscarried, and she had extensive exposure to grass. We suspected Q fever based on her occupation and tested for scrub typhus, Q fever, Lyme disease, leptospirosis, and *Brucella* using the blood sample that we had collected during her outpatient visit (November 14, 2017; Table 1).

IgM and IgG antibody titers against *Brucella abortus* were determined using a standard tube agglutination test. A microscopic agglutination test was conducted to rule out leptospirosis. For *O. tsutsugamushi* serology testing using whole blood, slides with a mixture of Kato, Karp, Gillium, and Boryong antigens were used. Serological test to detect Lyme disease was performed using Microgen Diagnostic, recomLine *Borrelia* IgG, IgM kit that uses recombinant *Borrelia burgdorferi* s.l. antigens (OspA, OspC, p100, VlsE, p39, p58, p18 (= DbpA; decorin-binding protein A), and p41 (flagellin)). Serological tests for Q fever such as immunofluorescence assay and Western blot assay (IFA and WB) were conducted using serum. Antibody tests were conducted at the Korea Centers for Disease Control and Prevention (Table 1) and showed positive results for *O. tsutsugamushi* and *C. burnetii*. Results were negative for brucellosis and leptospirosis. Echocardiography showed no abnormal findings. Considering the test results, we diagnosed the patient with a coinfection of Q fever and scrub typhus. She was prescribed doxycycline for additional 2 weeks, and her symptoms have improved.

To diagnose scrub typhus, positive results were obtained by nested polymerase chain reaction (PCR) targeting *O. tsutsugamushi*-specific 56-kDa outer membrane target (using Karp strain as a positive control).³ In addition, positive results were obtained for both nested and real-time PCR (qPCR) testing *C. burnetii* IS1111.^{5,6} However, negative results were observed for PCR tests against the cytidine triphosphate (CTP) synthase gene (*pyrG*) and outer surface protein A (*ospA*), which were conducted to diagnose Lyme disease.^{7,8} Reaction mixture with distilled water instead of template DNA was used as a negative control during each run of PCR. On sequencing, we confirmed the results as Boryong strain in the case of *O. tsutsugamushi* and *C. burnetii* in the case of Q fever.

Blood collected from the patient was later intraperitoneally injected into two BALB/c mice treated with cyclophosphamide (Sigma-Aldrich, St. Louis, MO). The mice were dissected on the 14th day after inoculation, and splenomegaly was observed in both mice (Figure 1). Enlarged spleens were

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TABLE 1
Follow-up antibody titer assessment of a 56-year-old patient diagnosed with *Coxiella burnetii* and *Orientia tsutsugamushi* coinfection

Sample collection day	Q fever				Scrub typhus				Lyme disease				Leptospirosis		Brucellosis	
	IFA IgG phase I/II	IFA IgM phase I/II	IS1111 N-PCR	IS1111 qPCR	IFA IgG	IFA IgM	56kDa N-PCR	IFA/WB IgG	IFA/WB IgM	pPrG N-PCR	ospA N-PCR	Microscopic agglutination test	STA IgG	STA IgM		
November 14, 2016	64/256	< 16/< 16	+ve	Ct 39.47	1,024	256	+ve	32/+ve	32/+ve	-ve	-ve	< 50	-	-		
November 28, 2016	32/128	< 16/< 16	-	-	2,048	2,048	-ve	-	-	-	-	< 50	-	-		
December 15, 2016	32/512	< 16/< 16	-	-	2,048	512	-	128/-ve	32/-ve	-	-	< 50	< 20	< 20		
February 2, 2017	64/512	< 16/< 16	-	-	1,024	256	-	16/-ve	< 16	-	-	< 50	< 20	< 20		
May 15, 2017	< 16/32	< 16/< 16	-	-	1,024	256	-	16/-ve	< 16	-	-	< 50	-	-		

IFA = immunofluorescent antibody; - = Not tested; -ve = negative; +ve = positive; PCR = polymerase chain reaction; STA = standard tube agglutination test; WB = Western blot.

collected, homogenized, and inoculated into confluent monolayers of L929 cells. Inoculated L929 cells were observed daily for 5 days and then passaged every 5–7 days to new monolayers of L929 cells. To identify *O. tsutsugamushi* infection, we performed 56-kDa nested PCR and immunofluorescent antibody (IFA) testing in every batch of passaged cells. We found more than 50% incidence of *O. tsutsugamushi* infection after five passages of inoculated L929 cells. Finally, we successfully isolated *O. tsutsugamushi* but could not isolate *C. burnetii*. A phylogenetic tree was constructed based on partial 56-kDa type-specific gene sequences of *O. tsutsugamushi*, using the patient's blood (buffy coat) and culture isolate sequences, along with the reference sequences obtained from the GenBank (Figure 2).

CONCLUSION

There have been reports of scrub typhus patients who exhibit coinfection with *Leptospira* spp.⁹ It is noteworthy that worldwide there has been only one other report of Q fever and scrub typhus coinfection from Taiwan.⁴ However, the Taiwanese study confirmed the presence of coinfection based on serological diagnosis alone.

In the presented study, we have reported the *C. burnetii* coinfection using the molecular diagnostic method. In addition, an IFA test against Lyme disease yielded titers of 32 for both IgM and IgG, and Western blot results were also positive. Although the antibody titers increased to 128 at follow-up, the Western blot of the specimen from the follow-up was negative. Follow-up tests conducted in February and May of 2017 yielded an antibody titer of 16 for IgG and a titer below 16 for IgM (Table 1).

In Q fever, *C. burnetii* proliferates in macrophages, allowing prolonged bacteremia. Higher levels of antibodies and immune complex formation can also occur. It has been reported that a high level of anticardiolipin antibody is associated with a rapid progression of acute Q fever.¹⁰ In our study while testing for Lyme disease, the patient's first serum showed a positive band on P100, P41 OspC against IgM but a negative band for IgG. However, any of the follow-up samples did not show positive results for IgG as well as IgM. Moreover, both PCR tests conducted to detect *Borrelia* spp. using an initial blood sample showed negative results (targeting the CTP synthase gene [*pyrG*] and outer surface protein A [*ospA*]). Because various nonspecific antibodies are known to be formed in Q fever patients, the patient's increased Lyme disease antibody titer is unlikely to have resulted from the actual presence of the disease, suggesting that the positive IFA test result was likely a false-positive result.¹¹

Coxiella burnetii can be isolated from at least nine different species of mites collected from rodents and birds, including *Allodermanyssus sanguineus* and *Hirstionyssus criceti*.¹² Therefore, although the role of mites as an arthropod reservoir of *C. burnetii* is unclear, additional studies are needed to assess the possibility that *C. burnetii* and *O. tsutsugamushi* coinfection can result from mite bites.

It is known that most patients with scrub typhus exhibit defervescence within 48 hours of appropriate antibiotic treatment. Therefore, if fever has not relieved within 48 hours, other diseases, such as malaria or dengue fever, should be suspected.¹⁰ Moreover, antibiotic treatment (doxycycline) reduces the rate of positive PCR test results to 10% within

FIGURE 1. (A) Splenomegaly with a diameter of approximately 2 cm (top) was observed in the mice infected with the patient's blood. For comparison, a normal mouse spleen with a diameter of 1.5 cm diameter is also shown (bottom). (B) Immunofluorescence antibody assay results of *Orientia tsutsugamushi*-infected cells. This figure appears in color at www.ajtmh.org.

4 days.¹³ In a controlled trial, we confirmed a 97.8% cure rate of mild-to-moderate scrub typhus after 5 days of doxycycline treatment.¹⁴ In this case, the patient's follow-up blood was negative for *O. tsutsugamushi* but her condition did not improve with the provided treatment; hence, additional investigation was performed toward coinfection identification.

In any case, this study has several limitations that are important to mention. We failed to isolate *C. burnetii* from a

mouse injected with the sample, and we did not obtain a definitive confirmation based on serology. Neither seroconversion nor a four-fold increase in IgG phase II titers in convalescence samples was documented. We could not exclude the possibility that the presence of IgG against *C. burnetii* was attributable to residual antibodies from a previous exposure. In addition, we could not exclude the possibility of *C. burnetii* DNA contamination during nested PCR.

FIGURE 2. Phylogenetic tree based on partial 56-kDa type-specific gene sequences of *Orientia tsutsugamushi*. Filled circles show the patient's blood (buffy coat) and culture isolate sequences. Reference sequences were obtained from GenBank.

In conclusion, this is the first study to identify scrub typhus and Q fever coinfection both molecularly and serologically. Moreover, we have the following suggestions for clinicians from regions in which scrub typhus and Q fever are endemic. Diagnosis should proceed carefully for a patient with eschar who is suspected of having scrub typhus. Attention should be paid to the possibility of coinfection with Q fever if the patient does not respond to doxycycline treatment, especially if he or she works in the livestock industry.

Received February 1, 2018. Accepted for publication February 12, 2019.

Published online March 25, 2019.

Acknowledgments: We thank research workers, such as Seung Hun Lee and Seon do Hwang, from the Korea Centers for Disease Control and Prevention (2014-NI52001-00).

Financial support: This study was supported by research funds from Chosun University Hospital 2016.

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