

# Cholestatic Hepatitis Caused by Tongyeong Strain of *Orientia tsutsugamushi*

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In Korea, the clinical manifestation of scrub typhus in humans appears to be severe in the northern regions of the country and mild in the southern areas. Studies on the geographic distribution of the serotype of *Orientia tsutsugamushi* revealed that the Boryong serotype is predominant in the southern Korean peninsula, whereas the Gilliam, Karp, and Gilliam-Karp serotypes prevail in the northern or central peninsula. We report a case of severe scrub typhus caused by the Japanese Gilliam type of *Orientia tsutsugamushi* in a 66-year-old woman from Tongyeong, a city located in the southern part of Korean peninsula. The patient was admitted to the hospital with fever on August 29th, 2001. Scrub typhus complicated by shock, pneumonitis, and encephalitis was diagnosed. Additionally, markedly elevated alkaline phosphatase levels was observed, which necessitated an ultrasonographic examination, which showed an ill-defined hyperechogenic mass on the right hepatic lobe. Liver biopsy showed cholestatic hepatitis and blood culture showed growth of *O. tsutsugamushi*. Gene sequencing of the amplicons of a polymerase chain reaction using primers for the amplification of the gene encoding the 56-kDa protein of *O. tsutsugamushi* revealed a nucleotide sequence that matched Yonchon strain of *O. tsutsugamushi*.

**Key Words :** *Orientia tsutsugamushi*, Scrub typhus, Cholestatic hepatitis, Genotype

## INTRODUCTION

There are many studies on the pathogenecity, epidemiology, clinical manifestations, and treatment of scrub typhus. However, the geographic distribution of the serotype of *Orientia tsutsugamushi* in Korea is not well known. Studies on the serotypes isolated from Korean patients reveal that the Gilliam, Karp, and Gilliam-Karp serotypes are predominant in the northern provinces of the Korean peninsula (Kyonggi, Chungchongbuk, and Kangwon provinces), whereas the Boryong serotype prevails in the southern provinces (Chungchongnam, Chollabuk, and Kyongsangnam provinces) (1-4). Because most of the

studies on the geographic distribution of the serotype have been carried out in the northern and central areas of the peninsula, it is difficult to apply these results to the southern part of the peninsula (Chollanam and Kyongsangbuk provinces). Despite these limitations, it has been presumed that Gilliam or Gilliam-Karp serotype infection in humans is rare in the central and southern regions of the country.

It has been thought that clinical manifestations would be different according to the serotypes or genotypes, but their relationship in humans has not been fully investigated. Severe scrub typhus can often be seen in Kyonggi and Kangwon provinces; however, it is thought to be rare in the southern areas of the peninsula. It is possible to presume that because of the similar geographic distribution of clinical severity and serotypes, the clinical manifestations of the Boryong serotype are mild and those of other serotypes, especially those of the Gilliam or Gilliam-Karp serotype, are severe. However, this hypothesis has

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yet to be proven.

The authors report a case of severe scrub typhus caused by the Japanese Gilliam type of *O. tsutsugamushi* in a patient residing in Tongyeong, a city located in the southern region of the Korean peninsula. This case (30-2001) has been cited in a previous report (5).

## CASE REPORT

### 1. Case

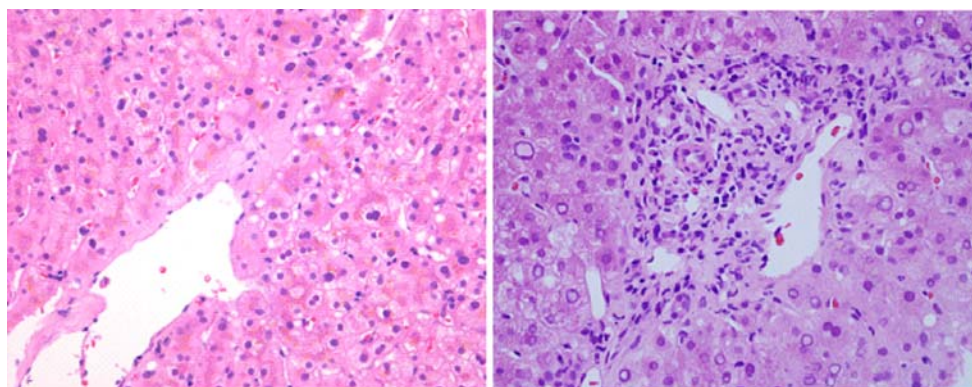
A 66-year-old woman was admitted through the emergency department with fever and a hepatic mass on August 29th, 2001. The patient was a farmer residing in Tongyeong, Kyongsangnam province, Korea, and had engaged in outdoor activities from the August 13th to 15th, 2001. On August 20th, fever developed, which was managed empirically as an upper respiratory infection at a local hospital. Laboratory examination performed at the local hospital revealed the following results: hemoglobin, 12.3 g/dL; white blood cell (WBC), 5,100/ $\mu$ L; platelet, 130,000/ $\mu$ L; aspartate aminotransferase (AST), 144 U/L; alanine aminotransferase (ALT), 100 U/L; alkaline phosphatase (ALP), 261 U/L; and total bilirubin, 0.4 mg/dL. The patient made another visit to the outpatient department on August 29th, due to persistent fever. Laboratory tests were conducted again and showed the following results: hemoglobin, 12.8 g/dL; WBC, 12,800/ $\mu$ L; platelet, 100,000/ $\mu$ L; AST/ALT, 250/95 U/L; ALP, 396 U/L; total bilirubin, 2.5 mg/dL; albumin, 3.5 g/dL; and blood urea nitrogen/creatinine (BUN/Cr), 35/1.2 mg/dL. Abdominal computerized tomography (CT) showed a hepatic mass with rim enhancement on the right hepatic lobe. On the same day, the patient was transferred to our hospital, located in Incheon, for further evaluation of the hepatic mass and fever.

Initial vital signs at the emergency department of our hospital were as follows: blood pressure, 80/50 mmHg; heart rate, 102/min; and body temperature, 36°C. Scrub typhus was suspected because of the eschar located in the right inguinal area along with whole body rashes. Abdominal ultrasonographic examination revealed an ill-defined hyperechogenic mass on the right hepatic lobe. Laboratory results showed the following results: hemoglobin, 12.7 g/dL; WBC, 12,000/ $\mu$ L; platelet, 49,000/ $\mu$ L; AST/

ALT, 235/79 IU/L; ALP, 1,333 IU/L; total bilirubin, 4.3 mg/dL; albumin, 2.7 g/dL; BUN/Cr, 43.0/2.2 mg/dL; C-reactive protein, 11.84 mg/dL; and erythrocyte sedimentation rate, 3 mm/hr. An electrocardiography showed tachycardia, supraventricular tachycardia, and ST segment depression; the levels of creatinine kinase and troponin-I were 40 IU/L and 0.24 mg/mL, respectively. Cardiac ischemia due to hypotension rather than myocardial infarction was diagnosed. Chest X-ray showed increased density on both lobes and arterial blood gas analysis revealed the following results: pH, 7.43; PCO<sub>2</sub>, 32.6; PO<sub>2</sub>, 52.8; HCO<sub>3</sub>, 21 mmol/L; and oxygen saturation, 85%.

Scrub typhus complicated by pneumonitis and hepatitis was diagnosed and myocarditis was initially suspected due to shock and the electrocardiographic abnormalities. The shock was managed with fluid and dopamine and subsequently the blood pressure rose to 120/70 mmHg within 2 h, and the urine output increased to 250 cc/h. After supplementation of oxygen, hypoxemia improved, and after 7 h, oxygen saturation rose to 97%. Doxycycline (300 mg/day) and hydrocortisone (300 mg/day) were administered, and a body temperature of 36.8°C was maintained. On the 2nd hospital day, the patient became drowsy. Therefore, central nervous system (CNS) involvement due to scrub typhus was suspected. The dose of doxycycline was increased to 400 mg/day. Pneumonic infiltration improved, but the drowsiness persisted. Antibody to *O. tsutsugamushi* was negative at 1:80 using a passive hemagglutination assay (GreenCross, Korea), but positive at 1:400 using an indirect immunofluorescent assay. On the 8th hospital day, vital signs were stable and pneumonitis was improved, but the patient continued to exhibit drowsiness. At this point, laboratory results were as follows: hemoglobin, 8.6 g/dL; WBC, 9,800/ $\mu$ L; platelet, 121,000/ $\mu$ L; AST/ALT, 105/91 IU/L; ALP, 778 IU/L; and total bilirubin, 3.0 mg/dL. Ultrasonography-guided liver biopsy was performed to exclude the possibility of liver abscess; it revealed cholestasis along with lymphocytes, plasma cells, and granulocytes in the portal space and sinusoids (Fig. 1).

On the 9th hospital day, mental status of the patient improved and finally normalized. The antibody test using a passive hemagglutination assay was rechecked and



**Figure 1.** Liver biopsy shows mild intraacinar cholestasis in the perivenular area (left) and mild widening of the portal area with lymphocytic, plasmacytic, and neutrophilic infiltration (right). (H&E,  $\times 400$ )

**Table 1.** Reactivity of Certain Monoclonal and Polyclonal Antibodies against Prototype strains of *O. tsutsugamushi* and the Present Isolate

Antibodies	Reactivity of <i>O. tsutsugamushi</i> strains tested					
	Boryong	Gilliam	Karp	Kato	Kuroki	Present isolate
Monoclonal antibodies						
FS15	+	-	+	+	+	-
Rb167	+	-	+/-	+	+	++
Rb1	+	-	-	-	++	-
Rb105	-	-	+/-	+	-	+
Rb130	-	-	+/-	+	-	-
Rb134	-	+	-	-	-	-
Rb136	+	+	+/-	++	+	+
Polyclonal antibodies						
Anti-Boryong	+++	-	+	+	++	++
Anti-Gilliam	++	+++	+	+	++	++

found to be negative at 1:80. On the 10th hospital day, CNS involvement by *O. tsutsugamushi* was confirmed through cerebrospinal fluid examination: red blood cell,  $1/\mu\text{L}$ ; WBC,  $7/\mu\text{L}$ ; protein, 132 mg/dL; and glucose, 87 (serum glucose 131) mg/dL. An electroencephalography revealed continuous slow waves. On the 11th hospital day, the patient was transferred to the general ward, and on the 15th hospital day treatments with doxycycline and hydrocortisone were discontinued. The patient was discharged on the 17th day after hospitalization. The patient was followed up at the outpatient department 7 days after discharge. Except for general weakness, the patient showed no specific symptoms and liver function tests were normal.

## 2. Determination of genotype

*O. tsutsugamushi* was cultured from blood samples.

The isolate was analyzed with FS15, a monoclonal antibody that reacts only to Boryong, Karp, Kato, Kuroki, and Kawasaki strains (6), and was found to be non-reactive. The pattern of reactivity of the isolate to other monoclonal and polyclonal antibodies was different from that of the known serotypes (Table 1).

For the amplification of the gene encoding the 56-kDa protein of *O. tsutsugamushi*, conventional polymerase chain reaction (PCR) was performed instead of nested PCR as described previously (7). The primers used for the amplification were primer 34 (5'-TCAAGCTTATTGCTA GTGCAATGTCTGC-3') and primer 11 (5'-CTAGGGAT CCCGACAGATGCACTATTAGGC-3'). Gene sequencing of the subsequent PCR product revealed 99% homology to Yonchon, Ikeda, 405S, Iwataki-1, and LP-1 strains (GenBank accession number FJ392859). Nucleotide sequence of the present isolate was registered in GenBank

(National Institutes of Health, Bethesda, MD, USA) as Tongyeong strain of *O. tsutsugamushi*. For the amplification of 16S ribosomal RNA, the primers used were 5'-AGTCGAACGAATTAATGCTGAG-3' and 5'-CTCTACCATACTCTAGCCTAAC-3'. The specific 568-bp product was obtained by PCR and nucleotide sequencing of the amplicon revealed 99% homology to Boryong, Ikeda, Kato, Karp, TH1817, Kawasaki, and Kuroki strains (GenBank accession number FJ392858). As a result, we were unable to characterize the genotype of the isolate by analysis of 16S ribosomal RNA.

## DISCUSSION

The patient suffered from a severe form of scrub typhus complicated by pneumonitis, encephalitis, and cholestatic hepatitis. Myocarditis was initially suspected due to the presence of shock, but 2 h after fluid infusion, the patient recovered from shock. Probably, the shock would have been due to transient hypovolemia which developed while the patient was being transferred from Tongyeong to Incheon. This case is interesting because of the following reasons: (1) cholestatic hepatitis was proven in scrub typhus, (2) Japanese Gilliam type infection occurred in Kyongsangnam province, and (3) it occurred during summer.

Abnormal liver function is well documented in scrub typhus (8). Previous reports mostly involved nonspecific hepatitis or granulomatous changes (9-13) and no report could be found on scrub typhus where not only aminotransferase but also alkaline phosphatase dramatically increased and where cholestatic hepatitis was demonstrated by liver biopsy. Whether the mass detected through abdominal CT was caused by scrub typhus or found incidentally cannot be determined; however, the fact that liver function normalized during the convalescent period supports the former hypothesis.

Determining the serotype or genotype of *O. tsutsugamushi* in clinical practice is rare because it is not essential for diagnosis or treatment. Studies on serotype or genotype were mainly performed when the presence of scrub typhus first became known in Korea.

Ten isolates, using serologic methods, from patients in

Chinhae were the Karp serotype (2), and 15 isolates from patients in Ulsan were the Boryong serotype and 1 was the Gilliam serotype (3). A study from Seoul, using PCR of biopsied eschar or rash, showed that 7 isolates were the Boryong-Kuroki serotype and 1 was the Karp serotype (4). A nationwide study involving 113 isolates from patients with scrub typhus that were analyzed using monoclonal antibodies revealed that the most common serotype was the Boryong serotype (88 isolates); most of these isolates were from individuals in the central and southern regions of the country. Of the remaining 25 isolates, 13 isolates were the Karp serotype, 9 isolates were the Gilliam serotype, and 3 were the Gilliam-Karp serotype; these 25 strains were from Kyonggi, Chungchongnam, Chungchongbuk, and Kangwon provinces (1).

Studies on serotype in wild rats using nested PCR revealed the following results: 7 wild rats from Chollanam province had Karp strain and 4 had Boryong strain (14). A nationwide study using the monoclonal antibodies revealed the Karp serotype to be the most common, followed by the Karp serotype variant; only 2 strains reacted to anti-Gilliam antibody (3.4%). Those 2 Gilliam strains were from rats captured in the Andong and Chungju cities. Boryong strain was not examined and was probably classified as the Karp serotype, the most similar strain to Boryong strain (15). Genotypes from DNA isolated from wild rodents and chigger mites revealed only the Boryong (78-83%) and Karp (12-16%) serotypes; Gilliam strain was not found (7).

The above studies in humans and in field rodents suggest that Gilliam or Gilliam-Karp strain infection is rare in the southern regions, but the present case shows that Japanese Gilliam type infection does occur in Tongyeong. In addition, considering the fact that the patient was admitted to the hospital in Incheon, where the patient's daughter resided, Japanese Gilliam type infection may be more common than was previously thought in southern areas of the Korean peninsula, including Tongyeong.

The nucleotide sequence of 56-kDa gene from the present case showed 99% homology to Yonchon, Ikeda, 405S, Iwataki-1, and LP-1 strains; hence, this isolate was classified as the Japanese Gilliam type (16). If classified by serologic methods, the Japanese Gilliam type is classi-

fied as the Gilliam-Karp serotype and may be erroneously classified as the Gilliam serotype if anti-Gilliam polyclonal antibodies are used; however, the gene sequence of the 56-kDa protein of the Japanese Gilliam type of *O. tsutsugamushi* is different from that of Gilliam prototype strain. Yonchon strain is the first one of the Japanese Gilliam type isolated in Korea (17); unfortunately, Tongyeong strain could not be compared to Yonchon strain.

Different virulence can be found in rats (7) and there is a possibility that different serotypes or genotypes give rise to different clinical manifestations in humans. The Gilliam serotype of *O. tsutsugamushi* is thought to be linked to severe scrub typhus, but this has yet to be proven by clinical research. One study conducted in Wonju showed no difference in severity between Gilliam serotype infection and infection by other serotypes (18); however, since the serotype in the study was determined by serologic reactions, the classification may be inaccurate. Our previous study showed that the Gilliam serotype could be divided into Gilliam prototype strain, which showed mild clinical manifestations, and Yonchon-like strain (Tongyeong strain in the present report), which showed severe clinical manifestations (5). These 2 strains cannot be distinguished by serologic reaction, so clinical difference could not be determined in the study conducted in Wonju. Further studies correlating the infection by Yonchon or Tongyeong strain determined by genotype and the severity of the illness are warranted.

Although there is ample evidence that scrub typhus can occur in summer in Korea, only 1 case has been reported (19). Atypical clinical manifestations were shown in the former report, but the present case showed typical manifestations of scrub typhus. The case occurred during summer in a patient residing in a coastal region, showing similar epidemiology and clinical manifestation to Japanese spotted fever (20). Unlike Japanese spotted fever, in the present case, a large eschar was present, there was no petechia, and a positive reaction using an indirect fluorescent antibody assay against *O. tsutsugamushi* was observed.

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