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Correlation between genotypes of *Orientia tsutsugamushi* and clinical characteristics of patients with scrub typhus in Guangzhou, China

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ABSTRACT

Objective: To explore the correlation between genotypes of *Orientia* (*O.*) *tsutsugamushi* and clinical characteristics of scrub typhus patients.

Methods: Clinical data of patients with scrub typhus admitted to different types of medical institutions in Guangzhou from September 2012 to December 2016 were collected using medical records. Demographic data, clinical manifestations, as well as hematological and biochemical indicators of patients infected with different genotypes were analyzed and compared.

Results: A total of 192 patients were included in this study, including 121 patients with Karp genotype of *O. tsutsugamushi* infection (63.0%), 36 patients with Gilliam genotype (19.0%), 23 patients with Kato genotype (12.0%), and 12 patients with TA763 genotype (6.0%) infection. The median value of albumin in patients with Karp genotype infection was significantly lower than that of Gilliam-infected patients (P=0.032). Patients with Karp genotype infection had a significantly longer hospital stay (9 days) than those with Gilliam genotype (7 days) (P=0.009) and Kato genotype infection (6 days) (P=0.005). Karp-infected patients also represented for the largest number of patients with complicated organ involvement (88/133, 66.2%). Furthermore, Karp-infected patients had higher risk of developing multiple organ dysfunction syndrome (18.2%) and requiring intensive care unit treatment (15.9%). Besides, patients with Gilliam genotype (8 days) and TA763 genotype infection (7.5 days) had shorter fever duration than those with Karp genotype (9 days) and Kato genotype (9 days) infection, respectively.

Conclusions: Genotypes of *Orientia tsutsugamushi* were associated with varying clinical manifestations, organ involvement, and treatment outcomes, suggesting that genotypes ranged in virulence.

KEYWORDS: Scrub typhus; *Orientia tsutsugamushi*; Genotype; Clinical characteristics

Significance

The correlation between genotypes of *Orientia tsutsugamushi* and disease severity is still unclear. In this study, we observed that Karp-infected patients had a higher frequency of various clinical symptoms, a wider range of organ system involvement, and a more severe condition. The clinical features of patients infected with Gilliam, Kato, and TA763 genotypes also showed distinct clinical characteristics, especially organ involvement preferences. These findings demonstrate that infecting genotypes play a role in the progression of the disease, and are correlated with the disease severity. These findings may provide clinical evidence on virulence differences of genotypes.

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1. Introduction

Scrub typhus (ST) is an acute infectious disease caused by *Orientia* (*O.*) *tsutsugamushi*, which is widely prevalent in the Asia-Pacific region[1]. *O. tsutsugamushi* can be transmitted to humans through the bite of chigger larvae living on rodents[2]. It has been estimated that more than 1 million new cases of ST occur every year, and approximately 1 billion people are at risk of *O. tsutsugamushi* infection around the world[3]. Antibacterial drugs with intracellular penetration, such as doxycycline, azithromycin, and chloramphenicol are commonly prescribed as clinical therapy for ST patients[4]. However, ST often manifests as an acute undifferentiated fever disease, and its presentation is similar to that of other zoonotic diseases, such as leptospirosis and dengue fever[5]. This similarity increases the likelihood of ST being overlooked or misdiagnosed, which may result in delays in providing appropriate treatments to the infected patients. Additionally, presence of an eschar at the bite site which can support the diagnosis of ST is not always observed in the patients[5]. Patients with severe ST may develop diffuse intravascular coagulation, acute respiratory distress syndrome, acute kidney injury, multiple organ dysfunction syndrome, and infectious shock[6]. Therefore, accurately assessing the risk of ST infection in patients during consultation is of great clinical significance since it determines whether doctors conduct further examination and timely treatment. Without timely treatment, the case fatality rate of ST could be estimated to be as high as 10%[7].

O. tsutsugamushi, a Gram-negative, obligate intracellular bacterium, is the etiological agent of ST. Based on the unique 56-kDa type specific antigen (*TSA56*), more than 40 genotypes of *O. tsutsugamushi* have been reported in recent decades[8,9]. Virulence differences of *O. tsutsugamushi* genotypes were reported in a few animal experiments and clinical studies[10–13]. Based on the susceptibility and pathoenic reactions of mice to *O. tsutsugamushi* strains, Nagano *et al.* divided several genotypes into three groups, including a high virulence group represented by Karp, Kato, and KN-3 genotypes, an intermediate virulence group represented by Gilliam genotype, and a low virulence group represented by Kuroki, Kawasaki, and KN-2 genotypes[10]. Some clinical studies observed that patients infected with various genotypes had different clinical manifestations. For example, Kim *et al.* reported that the incidence of eschar and rash differed between patients infected with Boryong and Karp genotypes of *O. tsutsugamushi*[11]. Wei *et al.* observed that patients infected with Kato genotype were more prone to splenomegaly and hepatomegaly[12]. Singla *et al.* reported that Karp genotype infection was associated with severe systemic manifestations[13]. Therefore, infecting genotypes may play a role in the development and severity of disease.

Guangzhou City is one of the megacities in China and is an epidemic area of ST with high incidence[14]. In recent years, the incidence of ST in Guangzhou has increased, the scope of infection has further expanded, and a variety of genotypes are prevalent, thus posing a major threat to human health and safety[15]. Therefore, this study collected data of ST patients in Guangzhou, summarized and analyzed their clinical features in hoping of providing data for improving the sensitivity of clinical diagnosis. This study also compared clinical manifestations among patients infected with different genotypes of *O. tsutsugamushi* and explored the correlation between genotypes and disease severity, hoping to provide more clinical evidence on virulence differences of genotypes, improve the sensitivity of clinical treatment.

2. Materials and methods

2.1. Data source

This retrospective observational study was carried out in Guangzhou, China from September 2012 to December 2016. Data were collected from the China Information System for Disease Control and Prevention, a professional web-based platform used to implement surveillance of infectious diseases. During the study period, up to 40 medical institutions of different sizes have reported ST cases to the system and these institutions located throughout 10 districts (including rural and urban areas) of Guangzhou City, some are tertiary hospitals and some are community hospitals. Professional doctors diagnosed and treated these patients, as well as gathered their whole blood specimens and medical records during their visit and hospitalization. DNA was extracted from blood specimens at the laboratory of the Guangzhou Center for Disease Control and Prevention, and *O. tsutsugamushi* genotypes were identified using nested polymerase chain reaction (nested PCR), DNA sequencing, and phylogenetic analysis (NCBI accession numbers: MT258651-MT258821). Details of the genotyping were published in our previous research[9]. Clinical data extracted from medical records were as follows: (1) patients' demographic information, medical history, and exposure history; (2) date of disease onset (the day when clinical signs or symptoms were observed), body regions presenting signs of a chigger bite, date of hospital admission, and date of discharge; and (3) clinical signs and symptoms, laboratory measurement indicators, and imaging findings.

2.2. Case definitions

According to the National Scrub Typhus Control and Prevention Guideline (2009) issued by the Chinese Center for Disease Control and Prevention[16], clinically diagnosed cases were defined as

people who had a travel history or field exposure history in ST-endemic areas and presented with fever and eschar/ulcer; or those who reported an unclear exposure history but presented with fever, eschar/ulcer and lymphadenopathy/skin rash during the ST-epidemic season. Laboratory-confirmed cases were defined as the clinically diagnosed cases with one of the following positive laboratory tests: Weil-Felix test; nested PCR; indirect immune fluorescence antibody assay; or isolation of *O. tsutsugamushi*. Cases that had other acute infectious diseases showing clinical manifestations similar to ST which are prevalent in Guangzhou like hemorrhagic fever with renal syndrome, dengue fever and leptospirosis, *etc.* were excluded.

Complicated ST cases were defined as presentation of any of the following situations[17–19]: (1) cardiac system involvement (arrhythmia, myocarditis, cardiac failure, *etc.*); (2) respiratory system involvement (acute upper respiratory tract infection, pneumonia, respiratory failure, *etc.*); (3) liver involvement (acute hepatotoxicity, hepatapostema, *etc.*); (4) renal involvement (acute renal insufficiency, acute renal failure, acute pyelonephritis, *etc.*); (5) gastrointestinal system involvement (acute upper gastrointestinal hemorrhage, acute gastroenteritis, *etc.*); (6) systemic circulatory system (including blood system) involvement (thrombocytopenia, sepsis, septicemia, *etc.*); (7) central nervous system involvement (dysphoria, convulsion, confusion, *etc.*); (8) multiple organ dysfunction syndrome (MODS), shock, or intensive care unit (ICU) admission; and (9) death.

2.3. Statistical analysis

All data were retrieved and proofread by a trained group of medical staff using Epidata software (version 3.1; The EpiData Association, Odense, Denmark). Categorical variables were summarized as frequencies or percentages. Continuous variables were expressed as median (IQR, interquartile range). *Chi*-square test or Fisher's exact test was used as appropriate to compare categorical variables of different genotype groups. Kruskal-Wallis *H* test was used to compare continuous variables of different genotype groups. Statistical analyses were performed using Microsoft Excel 2019 and SPSS version 25.0 (SPSS Inc., Chicago, IL, USA).

2.4. Ethical statement

This study was reviewed and approved by the Guangzhou Center for Disease Control and Prevention Ethics Committee (Number: GZCDC-ER[A]2017009). Written informed consent was obtained from all adult patients and guardians of children patients (below 18 years) before the collection of the case data, and all data were analyzed anonymously.

3. Results

3.1 Epidemiological characteristics among ST patients with different genotypes

In our previous study, a total of 906 whole blood specimens of clinically diagnosed ST hospitalized patients were collected from September 2012 to December 2016, of which 220 were positive based on nested PCR, showing a positive rate of 24.3%[9]. After excluding 28 patients without complete medical records, 192 patients were included in this study, including 121 patients with Karp-like strain infection (63.0%), 36 patients with Gilliam-like strain (19.0%), 23 patients with Kato-like strain (12.0%), and 12 patients with TA763-like strain infection (6.0%) (Figure 1). ST cases were reported in Guangzhou throughout the year and were mainly concentrated from May to November, with two peaks in May and October (Figure 2A). The distribution of ST patients in various administrative regions was relatively uneven, with the majority of patients localized in rural areas such as the Conghua District (68/192, 35.4%), Zengcheng District (55/192, 28.6%), and Huadu District (37/192,19.3%) (Figure 2B). The main occupations of patients were farmers and retirees, and most cases lived in rural areas. In terms of population distribution, the proportion of female patients (60.9%) was higher than that of male patients (39.1%). The median age of ST patients was 59 (50–67) years old, with patients over 60 years of age accounting for the highest proportion (52.1%). There was no significant difference in demographic baseline characteristics among patients infected with different genotypes (Table 1).

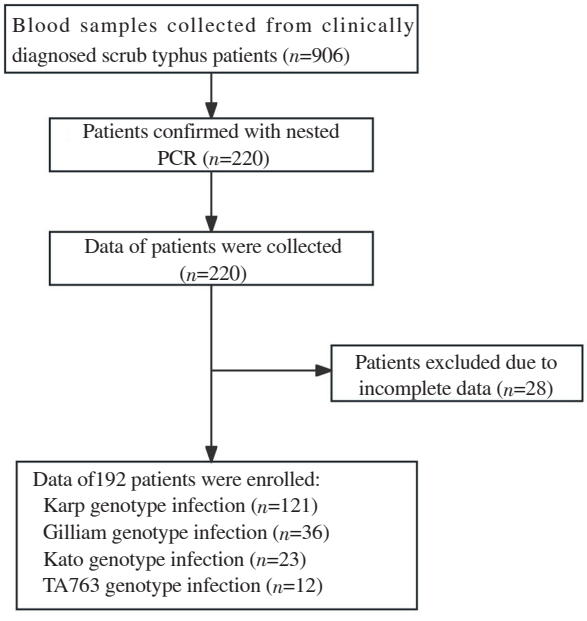

Figure 1. Flowchart of the study.

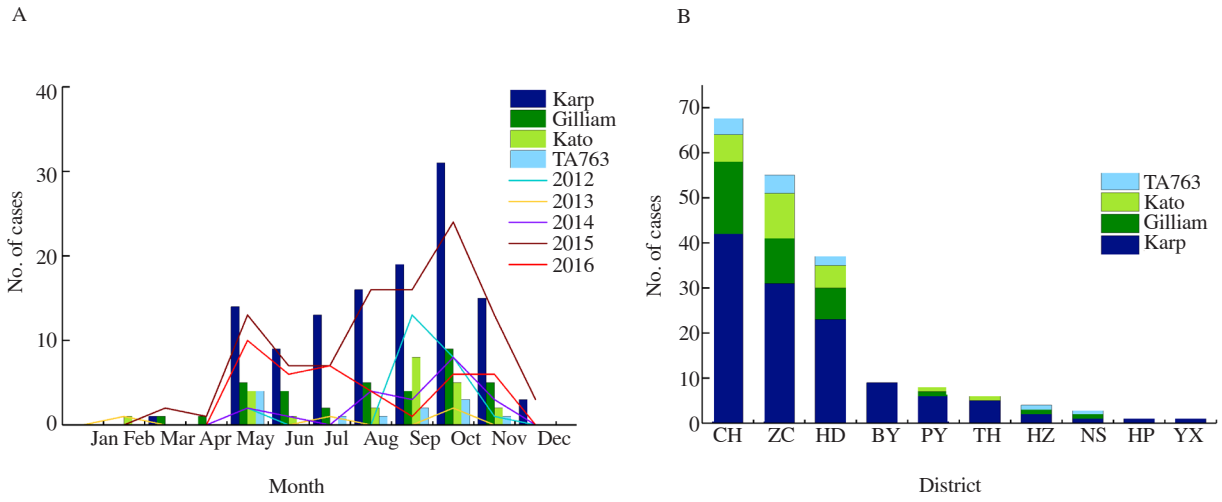

Figure 2. Temporal (A) and geographical (B) distribution of scrub typhus patients infected with different genotypes of *Orientia tsutsugamushi* in Guangzhou, during 2012-2016. Districts abbreviations are ZC, Zengcheng; CH, Conghua; HD, Huadu; BY, Baiyun; PY, Panyu; TH, Tianhe; HZ, Haizhu; NS, Nansha; HP, Huangpu; and YX, Yuexiu.

3.2. Clinical manifestations among ST patients with different genotypes

The median time from symptom onset to hospital admission for these ST patients was 6 (4-9) days, and the most common nonspecific clinical manifestation was fever. In this study, we found that all patients had fever symptom (100.0%), and the highest body temperature was approximately 39 ℃, with a certain persistence. The duration of fever refers to the time from the first day of fever to the day restoring normal body temperature. In this study, patients with Karp and Kato genotypes infection had longer median duration of fever (9 days), followed by those with Gilliam genotype (8 days) and TA763 genotype (7.5 days) (Table 1). In addition to fever, other common nonspecific clinical symptoms included headache (47.4%), feeble (45.8%), dizziness (27.1%), lymphadenopathy (20.8%), myalgias (17.7%), and peripheral edema (6.3%) (Table 1). Most patients manifested headache, feeble, dizziness, and lymphadenopathy when infected; however, peripheral edema was not observed in patients with Gilliam genotype, and no myalgia or peripheral edema was observed in patients with TA763 genotype (Table 1). The median length of hospital stay in patients with Karp genotype (9 days) was significantly longer than that of patients with Gilliam genotype (7 days) (P=0.009) and Kato genotype (6 days) (P=0.005) (Table 1 and Supplementary Table 1-4).

Common skin manifestations in ST patients were eschar (81.8%), ulcer (15.1%), and skin rash (7.8%) (Table 1). The presence of eschar often plays a decisive role in the clinical diagnosis of ST. Our study found that, compared with other genotypes, patients with Karp genotype had a higher proportion of presenting eschar at 85.1% (103/121), though the difference was not significant (Table 1). Furthermore, we also analyzed the distribution of eschar in patients of different sexes (Table 2). Most patients had only one eschar, but some patients had up to three eschars. The eschars mainly distributed in the armpits and external genitalia, accounting for 20.4% and 17.2%, respectively, while other common sites included the chest (16.6%), groin (16.6%), abdomen (14.6%), lumbar (14.0%), limbs (9.6%), buttocks (4.5%), and head and neck (3.2%). In female patients, eschars were mainly found in the chest (20.6%), groin (20.6%), armpit (19.6%), and abdomen (17.5%). The eschars of male patients were mainly distributed in the external genitalia (41.7%), armpit (21.7%), and lumbar (13.3%). The proportion of eschar in the external genitalia of male patients was significantly higher than that of female patients (41.7% *vs.* 2.1%; P<0.001).

We also observed cough (43.2%), expectoration (26.6%), and enlarged tonsils (7.3%) in these ST patients. However, there was no significant difference in respiratory manifestations among patients with different genotypes (Table 1). The most common gastrointestinal symptoms were anorexia (77.6%), nausea (22.4%), vomiting (14.6%), and abdominal pain (8.9%). Significant difference in vomiting was found among patients with different genotypes infection (P=0.036). No vomiting or abdominal pain was observed in patients with Kato and TA763 genotypes infection (Table 1). Chest radiographic abnormality (25.0%) were also common in ST patients. Other abnormal image findings included splenomegaly (12.0%) and pleural effusions (9.4%). However, there was no statistical difference in these image findings among patients with different genotypes (Table 1).

3.3. Laboratory findings among ST patients infected with different genotypes

As shown in Table 3, some hematological indicators in these

Table 1. Demographic baseline characteristics and clinical manifestations among scrub typhus patients stratified by infecting genotype.

Variables	Genotypes					*P* value
	Total (*n*=192)	Karp (*n*=121)	Gilliam (*n*=36)	Kato (*n*=23)	TA763 (*n*=12)	
Age, years, median (IQR)	59 (50–67)	60 (48–68)	58 (50–66)	59 (51–65)	62 (52–66)	0.878*
Age group, years						0.783†
0–14	4 (2.1)	3 (2.5)	0 (0.0)	1 (4.3)	0 (0.0)	
15–59	88 (45.8)	55 (45.4)	19 (52.8)	10 (43.5)	4 (33.3)	
≥60	100 (52.1)	63 (52.1)	17 (47.2)	12 (52.2)	8 (66.7)	
Sex, *n* (%)						0.916†
Female	117 (60.9)	75 (62.0)	21 (58.3)	13 (56.5)	8 (66.7)	
Male	75 (39.1)	46 (38.0)	15 (41.7)	10 (43.5)	4 (33.3)	
Occupation‡						0.161†
Farmers	60 (31.3)	37 (30.6)	15 (41.6)	4 (17.4)	4 (33.3)	
Retirees	59 (30.7)	35 (28.9)	10 (27.8)	9 (39.1)	5 (41.7)	
Children and students	5 (2.6)	4 (3.3)	0 (0.0)	1 (4.3)	0 (0.0)	
Others	24 (12.5)	20 (16.5)	2 (5.6)	0 (0.0)	2 (16.7)	
Unknown	44 (22.9)	25 (20.6)	9 (25.0)	9 (39.1)	1 (8.3)	
Residence						0.416†
Urban	24 (12.5)	19 (15.7)	2 (5.6)	2 (8.7)	1 (8.3)	
Rural	168 (87.5)	102 (84.3)	34 (94.4)	21 (91.3)	11 (91.7)	
Time from symptom onset to hospital admission, days, median (IQR)	6 (4–9)	6 (4–9)	6 (4–8)	7 (4–9)	6 (3–8)	0.375*
Length of hospital stay, days, median (IQR)	8 (6–10)	9 (7–12)	7 (5–8)	6 (4–8)	8 (5–9)	<0.001*
Non-specific manifestations						
Fever	192 (100.0)	121 (100.0)	36 (100.0)	23 (100.0)	12 (100.0)	NA
Maximum body temperature, ℃, median (IQR)	39.2 (39.0–39.7)	39.2 (39.0–39.8)	39.0 (38.8–39.6)	39.3 (39.0–40.0)	39.0 (38.9–39.3)	0.235*
Duration of fever, days, median (IQR)	9 (7–11)	9 (7–12)	8 (5–9)	9 (8–11)	7.5 (5–11)	0.019*
Headache	91 (47.4)	60 (49.6)	17 (47.2)	9 (39.1)	5 (41.7)	0.810†
Dizziness	52 (27.1)	35 (28.9)	6 (16.7)	8 (34.8)	3 (25.0)	0.402†
Feeble	88 (45.8)	58 (47.9)	18 (50.0)	6 (26.1)	6 (50.0)	0.262†
Myalgias	34 (17.7)	23 (19.0)	9 (25.0)	2 (8.7)	0 (0.0)	0.167†
Peripheral edema	12 (6.3)	11 (9.1)	0 (0.0)	1 (4.3)	0 (0.0)	0.203†
Lymphadenopathy	40 (20.8)	30 (24.8)	4 (11.1)	4 (17.4)	2 (16.7)	0.365†
Skin manifestations						
Skin rash	15 (7.8)	6 (5.0)	5 (13.9)	2 (8.7)	2 (16.7)	0.126†
Eschar	157 (81.8)	103 (85.1)	28 (77.8)	17 (73.9)	9 (75.0)	0.374†
Ulcer	29 (15.1)	14 (11.6)	7 (19.4)	5 (21.7)	3 (25.0)	0.229†
Respiratory manifestations						
Cough	83 (43.2)	48 (39.7)	15 (41.7)	14 (60.9)	6 (50.0)	0.289†
Expectoration	51 (26.6)	29 (24.0)	12 (33.3)	6 (26.1)	4 (33.3)	0.638†
Enlarged tonsils	14 (7.3)	10 (8.3)	2 (5.6)	1 (4.3)	1 (8.3)	0.966†
Gastrointestinal manifestations						
Anorexia	149 (77.6)	93 (76.9)	30 (83.3)	17 (73.9)	9 (75.0)	0.820†
Nausea	43 (22.4)	30 (24.8)	10 (27.8)	1 (4.3)	2 (16.7)	0.108†
Vomiting	28 (14.6)	23 (19.0)	5 (13.9)	0 (0.0)	0 (0.0)	0.036†
Abdominal pain	17 (8.9)	15 (12.4)	2 (5.6)	0 (0.0)	0 (0.0)	0.188†
Image findings						
Pleural effusion	18 (9.4)	13 (10.7)	3 (8.3)	2 (8.7)	0 (0.0)	0.853†
Chest radiographic abnormality	48 (25.0)	27 (22.3)	11 (30.6)	4 (17.4)	6 (50.0)	0.139†
Splenomegaly	23 (12.0)	15 (12.4)	5 (13.9)	2 (8.7)	1 (8.3)	0.959†

Abbreviations: IQR, interquartile range. NA, not available; Data are expressed as *n* (%) unless otherwise indicated; **P* value calculated by use of Kruskal-Wallis *H* test among genotype groups. †*P* value calculated by use of *Chi*-square test or Fisher's exact test among genotype groups. ‡Some columns do not add up to 100% because of rounding. All clinical manifestations (non-specific, skin, respiratory, and gastrointestinal) appeared before or at hospital admission, and all abnormal imaging results were reported after hospital admission.

Table 2. Distribution of eschar in scrub typhus patients stratified by gender [*n* (%)].

Location of eschar	Total (*n*=157)	Female (*n*=97)	Male (*n*=60)	*P* value
Head and neck	5 (3.2)	5 (5.2)	0 (0.0)	0.159
Armpit	32 (20.4)	19 (19.6)	13 (21.7)	0.845
Chest	26 (16.6)	20 (20.6)	6 (10.0)	0.086
Abdomen	23 (14.6)	17 (17.5)	6 (10.0)	0.254
Lumbar	22 (14.0)	14 (14.4)	8 (13.3)	0.821
Limbs	15 (9.6)	13 (13.4)	2 (3.3)	0.051
Groin	26 (16.6)	20 (20.6)	6 (10.0)	0.086
External genitalia	27 (17.2)	2 (2.1)	25 (41.7)	<0.001
Buttock	7 (4.5)	3 (3.1)	4 (6.7)	0.435

Notes: Data are *n* (%) until otherwise indicated. *P* value calculated by *Chi*-square test or Fisher's exact test between female and male patients.

Table 3. Laboratory indicators compared among scrub typhus patients stratified by infecting genotype.

Variables	Genotypes					*P* value
	Total (*n*=192)	Karp (*n*=121)	Gilliam (*n*=36)	Kato (*n*=23)	TA763 (*n*=12)	
Hematological indicators						
WBC count, 10^9/L	7.2 (5.2-9.5)	7.2 (5.1-9.5)	6.5 (4.9-8.8)	7.3 (5.6-8.6)	9.7 (5.9-11.4)	0.416
PLT count, 10^9/L	104.9 (72.8-149.8)	101.0 (62.0-139.0)	125.5 (98.8-158.0)	90.0 (62.0-113.5)	135.5 (77.9-159.8)	0.028
HGB, g/L	117.0 (106.0-131.0)	116.0 (101.0-132.0)	119.0 (106.0-130.9)	116.0 (106.0-126.3)	122.5 (109.8-128.9)	0.859
LYM count, 10^9/L	1.2 (0.7-2.1)	1.2 (0.6-2.0)	1.0 (0.7-2.2)	1.0 (0.7-1.6)	1.9 (1.0-2.4)	0.440
NEU count, 10^9/L	5.0 (3.6-6.9)	5.0 (3.4-7.0)	4.8 (3.4-6.1)	5.9 (4.3-6.9)	6.2 (4.1-8.3)	0.292
LYM percent, %	16.7 (11.3-26.9)	15.9 (9.4-27.3)	18.1 (12.8-26.4)	15.9 (12.4-25.0)	19.8 (16.3-24.8)	0.669
NEU percent, %	76.3 (63.7-83.1)	76.9 (62.2-85.4)	75.0 (62.6-81.4)	79.1 (70.3-81.7)	71.0 (61.4-77.1)	0.672
MON percent, %	5.8 (3.8-7.9)	5.4 (3.5-7.8)	7.2 (4.5-9.2)	6.1 (4.1-7.5)	4.9 (3.6-6.7)	0.302
HCT, %	35.1 (31.5-39.1)	34.4 (30.6-39.6)	35.7 (32.4-39.1)	35.0 (31.3-38.8)	37.0 (36.0-39.6)	0.230
MCV, FL	86.6 (81.5-90.5)	86.1 (79.4-90.0)	87.2 (83.7-91.2)	87.3 (84.1-91.9)	89.0 (82.6-94.1)	0.301
Biochemical indicators						
TBIL, μmol/L	13.6 (9.5-20.8)	13.3 (9.3-23.6)	16.7 (9.9-23.7)	14.7 (8.3-17.5)	12.2 (10.9-15.8)	0.617
AST, U/L	87.1 (57.0-131.0)	94.0 (57.0-133.0)	83.5 (65.5-116.0)	82.5 (57.8-136.0)	58.3 (41.8-127.8)	0.557
ALT, U/L	70.5 (44.0-107.5)	68.0 (44.0-106.0)	76.3 (45.0-97.0)	71.6 (48.3-110.0)	63.5 (29.4-147.8)	0.858
TP, g/L	61.0 (55.7-65.3)	60.6 (55.1-65.0)	62.5 (58.4-67.4)	58.6 (54.6-63.2)	63.2 (57.3-68.0)	0.175
ALB, g/L	32.8 (27.3-36.0)	31.8 (26.1-35.4)	34.9 (30.9-38.1)	31.0 (27.1-35.2)	34.0 (30.1-37.0)	0.026
GLB, g/L	29.0 (25.9-32.0)	29.1 (25.9-32.0)	28.8 (24.9-32.4)	28.0 (26.0-31.5)	29.1 (24.7-33.1)	0.986
LDH, U/L	627.0 (412.0-1050.0)	627.0 (395.0-1101.0)	806.0 (475.0-983.0)	521.0 (395.5-681.5)	607.0 (300.3-1034.8)	0.509
CK, U/L	104.6 (55.5-212.5)	104.8 (49.8-222.0)	116.0 (66.0-235.0)	102.5 (59.3-246.5)	77.5 (37.0-110.8)	0.293
ALP, U/L	98.0 (72.0-153.5)	102.0 (70.0-160.0)	98.0 (77.0-147.0)	96.5 (65.0-128.0)	87.5 (78.8-174.0)	0.630
GGT, U/L	81.0 (34.4-164.8)	80.0 (42.0-164.3)	53.0 (31.5-219.3)	88.5 (24.4-145.0)	92.0 (25.0-261.0)	0.606
CREA, μmol/L	82.4 (64.0-107.3)	83.4 (64.0-116.0)	79.5 (68.8-94.5)	83.0 (56.0-112.5)	74.0 (62.5-98.2)	0.803
Na, mmol/L	134.4 (131.0-137.3)	134.0 (131.0-138.5)	134.0 (133.0-137.3)	135.0 (130.3-136.4)	136.0 (134.0-137.0)	0.744
Cl, mmol/L	98.7 (96.3-102.5)	98.4 (96.0-103.4)	98.8 (96.2-100.2)	98.9 (97.0-100.3)	100.3 (96.3-103.0)	0.831
Ca, mmol/L	2.0 (1.9-2.2)	2.0 (1.9-2.1)	2.1 (2.0-2.2)	2.1 (1.9-2.2)	2.1 (2.0-2.2)	0.684

Notes: All laboratory measurements within 24h of hospital admission were presented as median (IQR, interquartile range). *P* value calculated by Kruskal-Wallis *H* test among genotype groups. Abbreviations: WBC, white blood cells; PLT, platelet; HGB, hemoglobin; LYM, lymphocyte; NEU, neutrophil; MON, monocyte; HCT, hematocrit; MCV, mean corpuscular volume; TBIL, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TP, total protein; ALB, albumin; GLB, globulin; LDH, lactate dehydrogenase; CK, creatine kinase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; CREA, creatinine; Na, sodium; Cl, chlorine; Ca, calcium.

Table 4. Complicated scrub typhus patients stratified by infecting genotype.

Variables	Genotypes					*P* value
	Total (*n*=133)	Karp (*n*=88)	Gilliam (*n*=23)	Kato (*n*=14)	TA763 (*n*=8)	
Cardiac system involvement	21 (15.8)	16 (18.2)	3 (13.0)	2 (14.3)	0 (0.0)	0.619
Arrhythmia	15 (11.3)	10 (11.4)	3 (13.0)	2 (14.3)	0 (0.0)	0.902
Myocarditis	4 (3.0)	4 (4.6)	0 (0.0)	0 (0.0)	0 (0.0)	0.820
Cardiac failure	2 (1.5)	2 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Respiratory system involvement	91 (68.4)	59 (67.1)	14 (60.9)	11 (78.6)	7 (87.5)	0.644
Acute upper respiratory tract infection	11 (8.3)	7 (8.0)	2 (8.7)	0 (0.0)	2 (25.0)	0.225
Acute bronchitis/Acute exacerbation of chronic bronchitis	22 (16.5)	16 (18.2)	2 (8.7)	4 (28.6)	0 (0.0)	0.318
Acute exacerbation of COPD	3 (2.3)	1 (1.1)	0 (0.0)	1 (7.1)	1 (12.5)	0.114
Pneumonia	56 (42.1)	36 (40.9)	10 (43.5)	6 (42.9)	4 (50.0)	0.959
ARDS	1 (0.8)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Respiratory failure	1 (0.8)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Liver involvement	75 (56.4)	47 (53.4)	15 (65.2)	7 (50.0)	6 (75.0)	0.692
Acute hepatotoxicity	75 (56.4)	47 (53.4)	15 (65.2)	7 (50.0)	6 (75.0)	0.692
Hepatapostema	1 (0.8)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Renal involvement	17 (12.8)	12 (13.6)	2 (8.7)	2 (14.3)	1 (12.5)	0.868
Acute renal insufficiency/Acute kidney injury	8 (6.0)	6 (6.8)	1 (4.4)	0 (0.0)	1 (12.5)	0.433
Acute renal failure	2 (1.5)	2 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	0.756
Acute pyelonephritis	1 (0.8)	0 (0.0)	1 (4.4)	0 (0.0)	0 (0.0)	0.358
Urinary system infection	6 (4.5)	4 (4.6)	0 (0.0)	2 (14.3)	0 (0.0)	0.271
Gastrointestinal system involvement	18 (13.5)	14 (15.9)	4 (17.4)	0 (0.0)	0 (0.0)	0.255
Acute upper gastrointestinal hemorrhage	2 (1.5)	2 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Acute gastroenteritis/Acute exacerbation of chronic gastroenteritis	16 (12.0)	12 (13.6)	4 (17.4)	0 (0.0)	0 (0.0)	0.321
Systemic circulatory system (including blood system) involvement	32 (24.1)	25 (28.4)	5 (21.7)	2 (14.3)	0 (0.0)	0.068
DIC	1 (0.8)	1 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0.899
Thrombocytopenia	5 (3.8)	4 (4.6)	0 (0.0)	1 (7.1)	0 (0.0)	0.639
Sepsis	4 (3.0)	3 (3.4)	1 (4.4)	0 (0.0)	0 (0.0)	0.82
Septicemia	2 (1.5)	1 (1.1)	1 (4.4)	0 (0.0)	0 (0.0)	0.602
Hypoproteinemia	23 (17.3)	19 (21.6)	3 (13.0)	1 (7.1)	0 (0.0)	0.083
Central nervous system involvement	10 (7.5)	10 (11.4)	0 (0.0)	0 (0.0)	0 (0.0)	0.164
MODS	16 (12.0)	16 (18.2)	0 (0.0)	0 (0.0)	0 (0.0)	0.016
ICU	14 (10.5)	14 (15.9)	0 (0.0)	0 (0.0)	0 (0.0)	0.036
Shock	9 (6.8)	8 (9.1)	1 (4.4)	0 (0.0)	0 (0.0)	0.644
Death	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA

Abbreviations: COPD, chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome; DIC, disseminated intravascular coagulation; MODS, multiple organ dysfunction syndrome; ICU, intensive care unit; NA, not available. Data are expressed as *n* (%) unless otherwise indicated. *P* value calculated by Chi-square test or Fisher's exact test among genotype groups.

ST patients decreased, such hemoglobin (HGB) and lymphocyte (LYM) percentage, while others increased like neutrophil (NEU) percentage. Abnormal biochemical indicators including aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and γ–glutamyl transpeptidase (GGT) increased, while the total protein (TP), albumin (ALB), sodium (Na), and calcium (Ca) decreased. Specifically, LDH increased the most, with a median value of 627.0 (412.0–1 050.0) U/L, which was more than twice the normal value (109.0-245.0 U/L) (Table 3).

No significant association between genotypes and abnormal hematological indicators were observed. As for the abnormal biochemical indicators, significant difference in the median value of ALB was found among patients with Kato genotype, Karp genotype, TA763 genotype and Gilliam genotype infection as shown in Table 3 (P=0.026). Pairwise comparisons revealed that the median value of ALB in patients with Karp genotype was significantly lower than those with Gilliam genotype infection (P=0.032) (Supplementary Table 1-4). Among other abnormal biochemical indicators, the median value of AST was highest in patients with Karp genotype, ALT and LDH in patients with Gilliam genotype, and GGT in patients with TA763 genotype; TP was lowest in patients with Kato genotype, although these differences were not statistically significant (Table 3).

3.4. Complicated ST cases

A total of 133 ST patients were diagnosed as complicated ST cases, including 88 Karp-infected patients (66.2%), 23 Gilliam-infected patients (17.3%), 14 Kato-infected patients (10.5%), and 8 TA763-infected patients (6.0%) (Table 4). In terms of specific organ involvement, respiratory system involvement in ST patients accounted for the highest proportion (91/133, 68.4%), including pneumonia (56/133, 42.1%) and acute bronchitis/acute exacerbation of chronic bronchitis (22/133, 16.5%). This was followed by liver involvement such as acute hepatotoxicity (75/133, 56.4%). ST patients also presented systemic circulatory system (including the blood system) involvement (32/133, 24.1%), cardiac system involvement (21/133, 15.8%), gastrointestinal system involvement (18/133, 13.5%), renal involvement (17/133, 12.8%), and central nervous system involvement (10/133, 7.5%) (Table 4). It was shown that Karp-infected patients had the highest proportion of systemic circulatory system (including blood system) involvement (28.4%), cardiac system involvement (18.2%), and central nervous system involvement (11.4%), although the differences were not significant. Furthermore, only patients with Karp genotype infection had central nervous system involvement (Table 4). Gilliam-infected patients had a high probability of gastrointestinal system involvement (17.4%). Kato-infected patients had slightly higher risk of getting renal involvement (14.3%) than those with other genotypes, but no gastrointestinal system involvement was observed in these patients. The most common organ involvements observed in TA763-infected patients were respiratory system involvement (7/8, 87.5%) and liver involvement (6/8, 75.0%) (Table 4). In terms of disease severity, no patient died; however, 12.0% (16/133) patients developed multiple organ dysfunction syndrome, 10.5% (14/133) required ICU care, 6.8% (9/133) experienced shock (Table 4).

4. Discussion

This study showed Guangzhou City, one of the main epidemic areas of ST in mainland China, had two major incidence peaks in May and October during 2012 and 2016. The reason might be that May and October are months for intensive agricultural activities in rural areas. As young adults migrate to urban areas for work, the left-behind elderly become the predominant labor force engaged in agricultural activities. Long-term outdoor agricultural work increases the risk of exposure and infection for elderly farmers. Furthermore, as the hot temperature drops after October, people spend more time on outdoor entertainment activities, which also increases the risk of being bitten by chigger mites. These features also partially explained the occupational preferences of ST patients, namely that farmers (especially elderly farmers) and retirees were at high risk of ST infection, which was consistent with a previous study[15].

In this study, we summarized and analyzed the pathogenic reactions of *O. tsutsugamushi* genotypes in the human. First, our conclusion about fever as the most common clinical symptom of ST was consistent with previous research findings in different regions[5, 20]. Our study further analyzed the length of fever duration in ST patients and found that patients with Karp genotype had the longest fever duration. Eschar is a cutaneous hallmark of ST. When chigger mite larvae bites the human body, it forms a primary papule on the bite site, which then enlarges and develops an ulcer with central necrosis, and finally turns into a black eschar[6]. The detection rate of eschar varied from 1% to 97% in different geographic areas and studies[5, 21,22]. This variation may be attributed to the difficulty in identifying eschar in dark-skinned individuals. Eschar was more commonly identified on Caucasian and East Asian patients[23,24]. The location of eschar may also affect the detection rate. Our study found that some eschars appeared on private areas such as armpit and external genitalia, which could pose challenges for clinical diagnosis. Furthermore, the presence of eschar in ST patients may be related to different genotypes of *O. tsutsugamushi* infection. Kim *et al.* found a significant difference in the incidence of eschar between patients infected with Boryong and Karp genotypes of *O. tsutsugamushi* (97.0% *vs.* 73.7%)[11]. However, significant difference

was not found in this study, even though patients with Karp genotype infection accounted for the highest proportion of patients with eschar. Exploration about association between particular *O. tsutsugamushi* strains infection and presence of eschar needs further studies.

We also observed that strain variations may contribute to varying clinical manifestations, organ involvement and treatment outcomes. For example, the Karp genotype infection was more likely to involve cardiac system, kidney system, gastrointestinal system, systemic circulatory system (including blood system), and central nervous system, *etc.*, and could invade multiple organ systems simultaneously, causing multiple organ dysfunction syndrome or shock. In other words, patients infected with Karp genotype were more likely to experience severe situations than those with other genotypes. Similar to our conclusion, a study from India showed that Karp genotype was the most prevalent genotype related to acute kidney injury in eastern India[25], and another study of pediatric ST showed that among the 14 cases studied, Karp was the most common genotype with a high incidence of multiple organ dysfunction[13]. Furthermore, our findings also revealed that the Gilliam, TA763, and Kato genotypes were more likely to invade the gastrointestinal, respiratory system, cause symptoms of liver, and kidney impairments, respectively. These organ involvement preferences may be caused by the virulence of the different strains of *O. tsutsugamushi*.

The difference in strain virulence may be related to differences in the antigenicity of the *TSA56* gene[8], which is a key point for genotyping *O. tsutsugamushi*[26]. This gene is considered to be full of point mutations and gene recombination, resulting in multiple antigenic variants of *O. tsutsugamushi*[9,27]. A recent phylogenetic analysis revealed considerable differences in the TSA56 gene sequences of different genotypes[9]. Due to the important role of *TSA56* gene in the adhesion to and invasion of *O. tsutsugamushi* into host cells[28,29], differences in *TSA56* gene may affect the recognition of organ receptors and further organ invasion by different *O. tsutsugamushi* genotypes. This might be connected to our study's finding about the organ involvement preference after infection with certain genotypes. Furthermore, the virulence of different strains may influence the ability to escape phagosomes and survive killing in the host cytoplasm[30], resulting in varied disease severity. These differences in disease progression and outcome reflected virulence differences of *O. tsutsugamushi* genotypes. Our study on the correlation between genotypes and disease progression and outcome can provide clinical data for further comparing similarities and differences of sequences of different genotypes, assisting in identifying gene segments that determine virulence. On the other hand, more severe clinical symptoms were observed in patients with Karp genotype in this study; however, no significant difference was observed in organ involvement and disease severity among patients with Gilliam, Kato, and TA763 genotypes, which was somewhat different from the virulence grouping of genotypes used by other researchers[10,31]. The difference in the recipient of infection (mouse or human) may be an influencing factor because researches on the virulence of different *O. tsutsugamushi* genotypes were mainly performed in animal experiments[30–32]. More research on pathogenic responses in the human is required to further confirm and categorize the virulence of different genotypes, which could help researchers better understand *O. tsutsugamushi* and ST. Additionally, Tang *et al.* previously observed that a patient in China was co-infected with at least two different strains of *O. tsutsugamushi* and experienced pulmonary involvement and liver dysfunction[33]. All patient included in this study were infected with single *O. tsutsugamushi* strain. Therefore, more data is required to verify the impact of mixed infections of different strains on disease severity.

This study is subject to some limitations. First, only hospitalized patients were included in the analysis, and outpatients were not further investigated. Second, as this study only analyzed ST patients in Guangzhou, the application of our results to other regions may be limited in view of the geographical differences in the distribution of genotypes. Moreover, in order to study deeper and reflect the results better, our research should be evaluated at a large scale with more cases in the future.

Conflict of interest statement

We declare that we have no competing financial interests.

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Authors' contributions

JLL participated in the study design, literature search, statistical analysis, and manuscript writing, editing and reviewing. ZYZ participated in literature search, statistical analysis, and manuscript writing. HYC and XT contributed to data acquisition and methodology. XWW and SYC contributed to funding acquisition. XQZ and JXX participated in manuscript editing and reviewing. LQF, LZ and YHW were involved in project administration and supervision.

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