

Abstract of Dissertation submitted by QIANG XU

**Title:** Clinical Factors Associated with SFTS Diagnosis and Severity in Cats

**Japanese title:** 猫の SFTS 診断と重症度に関連する臨床的要因

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**Introduction:**

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne disease caused by the SFTS virus (SFTSV), a member of the genus *Bandavirus* in the *Phenuiviridae* family. Initially identified in China in 2010 and subsequently detected in South Korea and Japan in 2013, these three countries report annual cases and are considered endemic regions. More recently, SFTSV has also been found in other Asian countries, including Vietnam, Thailand, Pakistan, and Myanmar, indicating a potential expansion of the endemic region. The mortality rate for SFTS varies from 5% to 30%, with the elderly facing a higher risk of fatal outcomes. Clinical manifestations include acute high fever with thrombocytopenia and leukopenia. Presently, there are no antiviral drugs or vaccines available for the treatment of SFTSV.

The primary mode of SFTSV transmission is through tick bites, with *Haemaphysalis longicornis* identified as the main vector. In addition to tick bites, SFTSV transmission can occur through contact with bodily fluids from infected individuals, and there have been reports of transmission from companion animals to humans. The incidence of SFTS in cats in Nagasaki is notably high in Japan, where many cats collectively owned and cared for by community members without designated owners. This implies cats could be a significant risk factor for SFTSV transmission within the surrounding community. Nevertheless, comprehensive information regarding the clinical presentations, laboratory parameters for SFTS diagnosis, and prognostic factors in cats is currently lacking. In this investigation, we identified biomarkers for SFTSV infection through the analysis of samples from cats with suspected SFTS and devised a scoring model to predict SFTSV infection. Implementation of this model may aid in safeguarding owners, community members, veterinarians, and healthcare professionals against the risk of SFTSV infection in cats.

**Materials and Methods:**

Between March 2018 and January 2024, a total of 221 cats with suspected SFTS were recruited from diverse animal hospitals in Nagasaki Prefecture, Japan. Serum and swab specimens were gathered from these felines. After excluding cases lacking adequate clinical data, 187 cases were included in the final analysis.

Real-time quantitative polymerase chain reaction (RT-qPCR) was utilized to detect SFTSV RNA. RNA extraction from the samples was carried out using Isogen-LS, followed by RT-qPCR conducted with the One-Step PrimeScript RT-PCR Kit on a 7500 Real-Time RT-PCR System. Specific primers and probes were designed based on the consensus genome sequence of the RNA-dependent RNA polymerase (RdRp)-coding region in the SFTSV-L segment.

For samples with SFTSV RNA copy numbers exceeding  $10^5/5 \mu\text{L}$ , virus isolation was performed on serum specimens using Vero E6 cells. SFTSV was successfully isolated from 16 out of the 27 cat samples. Full-length coding sequences of the SFTSV L/ M/ S segments were acquired through Sanger sequencing with specific primer sets, and phylogenetic analysis of the newly isolated SFTSV was conducted using MEGA11.

Comprehensive clinical and epidemiological data were gathered via detailed data sheets associated with the samples. These sheets encompassed information on gender, age, weight, clinical manifestations, and laboratory findings. Statistical analysis was carried out using R 4.2.2 software. The Wilcoxon rank-sum test was applied to compare continuous variables between SFTSV-positive and SFTSV-negative groups, while Fisher's exact test was employed for categorical variables. Pearson correlation coefficients were computed to evaluate relationships between continuous variables, and Spearman correlation coefficients with false-discovery rate correction were used for correlation matrix analysis. A scoring model was developed for predicting SFTSV infection, with p-values below 0.05 considered statistically significant.

### **Results:**

Of the 187 cases analyzed, no significant differences were observed between SFTSV-positive and SFTSV-negative groups in terms of sex, history of tick bites, or rearing environment. However, clinical signs such as vomiting were more frequently observed in SFTSV-positive cases, and the mortality rate was higher compared to the SFTSV-negative group. In SFTSV-positive cases, heavier body weight, increased red blood cell (RBC) counts, and elevated levels of aspartate aminotransferase (AST) and total bilirubin (TBil) were significant findings, whereas white blood cell (WBC) and platelet (PLT) counts were decreased. Among fatal SFTSV-positive cases, higher levels of alanine aminotransferase (ALT), AST, and serum RNA were noted.

The analysis revealed that viral RNA levels in SFTSV-positive cases correlated with several clinical and laboratory parameters. Body weight, RBC count, WBC count, Plt count, AST, and TBil were identified as useful biomarkers for SFTS diagnosis, while ALT, AST, and serum SFTSV RNA levels were associated with clinical outcomes. Phylogenetic analysis based on the SFTSV M segment indicated the relationship between newly isolated SFTSV strains and those from previous studies, demonstrating that the isolates were mostly classified into J1 clades, with no clear association between outcome and viral genetic variation.

### **Discussion:**

This study provides comprehensive data on the clinical features and laboratory parameters associated with SFTS in cats. The findings highlight that heavier body weight, increased RBC counts, and elevated AST and TBil levels are indicative of SFTSV infection, while higher ALT, AST, and serum RNA levels are linked to poorer clinical outcomes. These biomarkers could facilitate the diagnosis and prognosis of SFTS in cats, aiding veterinarians in managing the disease more effectively.

Moreover, the phylogenetic analysis underscores the genetic diversity of SFTSV strains but does not establish a direct correlation between viral genetic variations and disease outcomes. This insights from this study into the clinical and molecular aspects of feline SFTS are crucial for mitigating the zoonotic risk posed by SFTSV. This, in turn, protects cat owners, community members, and veterinary professionals from potential infections. The identification of reliable biomarkers for SFTS will enhance diagnostic accuracy and treatment efficacy, ultimately contributing to better public health outcomes in regions where SFTS is endemic.