Shedding and Transmission Modes of Severe Fever With Thrombocytopenia Syndrome virus in a Ferret Model

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Abstract

Although human-to-human transmission of severe fever with thrombocytopenia syndrome virus (SFTSV) via direct contact with body fluids has been reported, the role of specific body fluids from SFTSV-infected hosts has not been investigated in detail. To demonstrate the virus transmission kinetics in SFTSV-infected hosts, we adapted the ferret infection model and evaluated the virus shedding periods, virus titers, and transmission modes from various specimens of infected ferrets.

The qRT-PCR demonstrated that large amounts of infectious SFTSV are shed through nasal discharge, saliva, and urine from SFTSV-infected ferrets. Virus could be detected from 2 dpi and persisted until 12 dpi in these specimens, compared with the relatively short virus-shedding period in sera. Further, transmission studies revealed that SFTSV can be transmitted to close direct and indirect contact naïve animals through various mediums, especially through contact with serum and urine. Further, ferrets contacted with human urine specimens from SFTSV-positive patients were successfully infected with SFTSV, suggesting that urine specimens could be a source of SFTSV infection in humans.

Our results demonstrate that the SFTSV can be shed in various body fluids for more than 12 days and that these specimens could be a source for direct or indirect transmission through close personal contact.

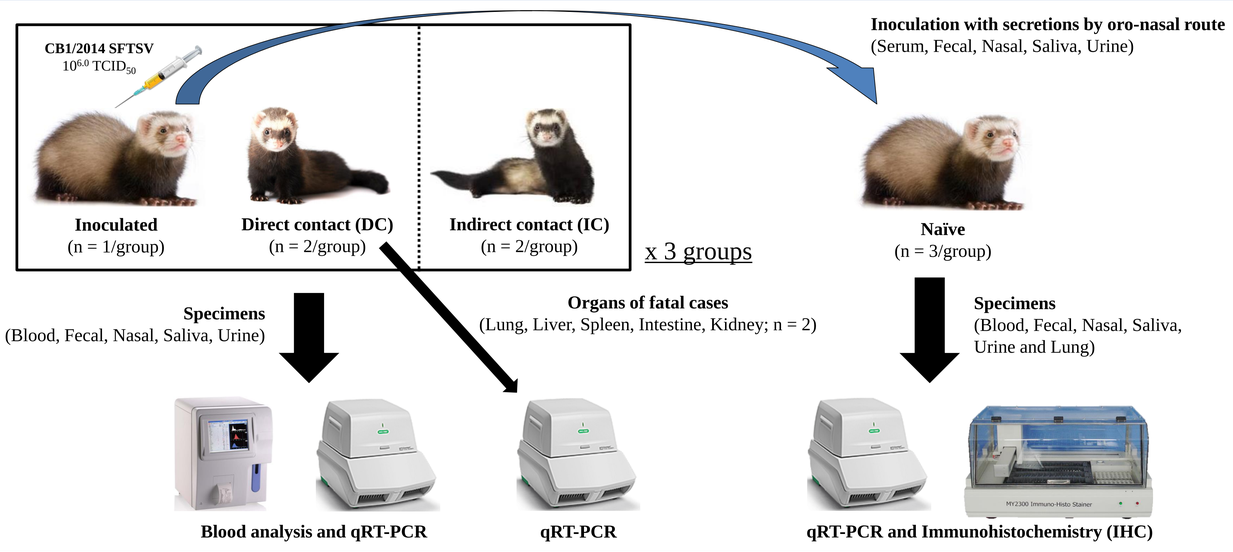
Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging zoonotic infectious disease caused by SFTSV, a novel member of the family *Phenuiviridae*. Since the first report of this virus in China in 2009, SFTS has mainly been found in China, Japan, and South Korea, with growing annual incidence and high case fatality rates. SFTSV is believed to be maintained in nature by an enzootic tick–animal cycle. Of the tick species, *Haemaphysalis longicornis* are implicated as a main vector of SFTSV.

Although SFTSV infection in humans is believed to be predominantly mediated through bites from virus-infected ticks, the first human-to-human transmission of SFTSV through contact or exposure to patient blood was reported in 2012. Since then, possible human-to-human transmissions of SFTS have been reported in families, co-residents of villages, and even in the hospital setting. Further, some secondarily infected patients died with high viral loads and clinical symptoms, including high fever and low platelet counts. Investigation of a cluster of SFTS cases provided evidence of person-to-person transmission through contact with blood from an index patient with a high serum virus load. However, detailed information regarding the kinetics of virus shedding in various body fluids and the manner of SFTSV transmission in vivo is quite limited due to the previous lack of an animal infection model.

Therefore, in this study, we performed direct and indirect contact transmission studies using a ferret model, which was recently characterized as a suitable model for SFTSV human infection. In this model, we attempted to trace virus transmission and identify the specimens most likely mediating transmission from infected hosts. The virus-shedding periods and viral loads of various specimens were comparatively analyzed using real-time reverse transcription polymerase chain reaction (RT-PCR). In addition, the presence of infectious virus in collected ferret and human specimens was demonstrated through infection in sentinel ferrets.

Design and Results



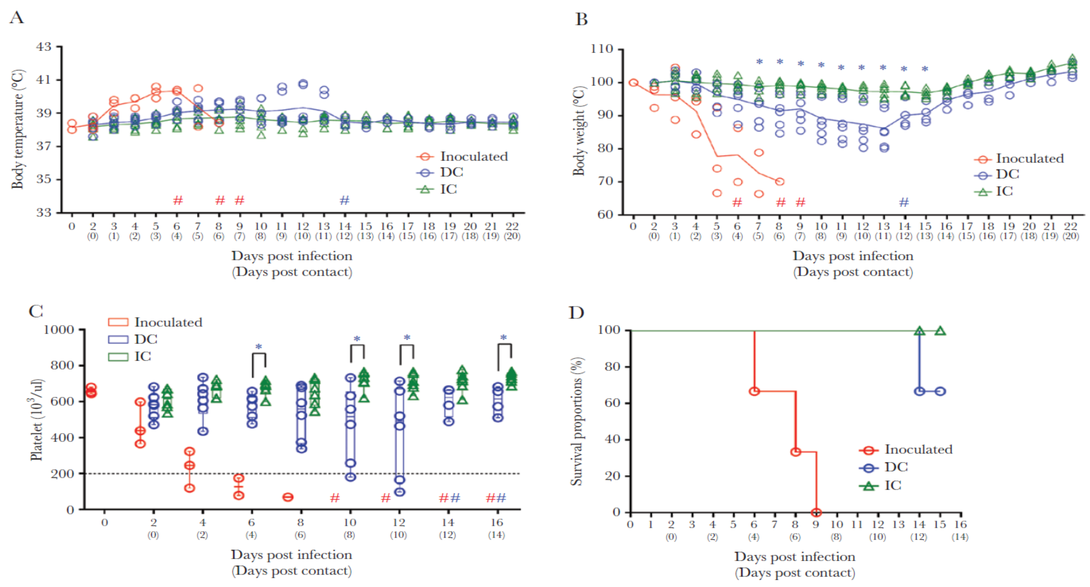


Figure 1. Clinical symptoms in ferrets after inoculation with CB1/2014 severe fever with thrombocytopenia syndrome virus. Three ferrets were inoculated intramuscularly with $10^{6.0}$ $TCID_{50}$ of virus, and direct contact (DC) and indirect contact (IC) ferrets (n = 6/group) were introduced into the cage at 2 dpi. The number indicates a reduced number of samples collected due to death of ferrets in this group. Data are presented with the horizontal dotted line as the minimum threshold values.

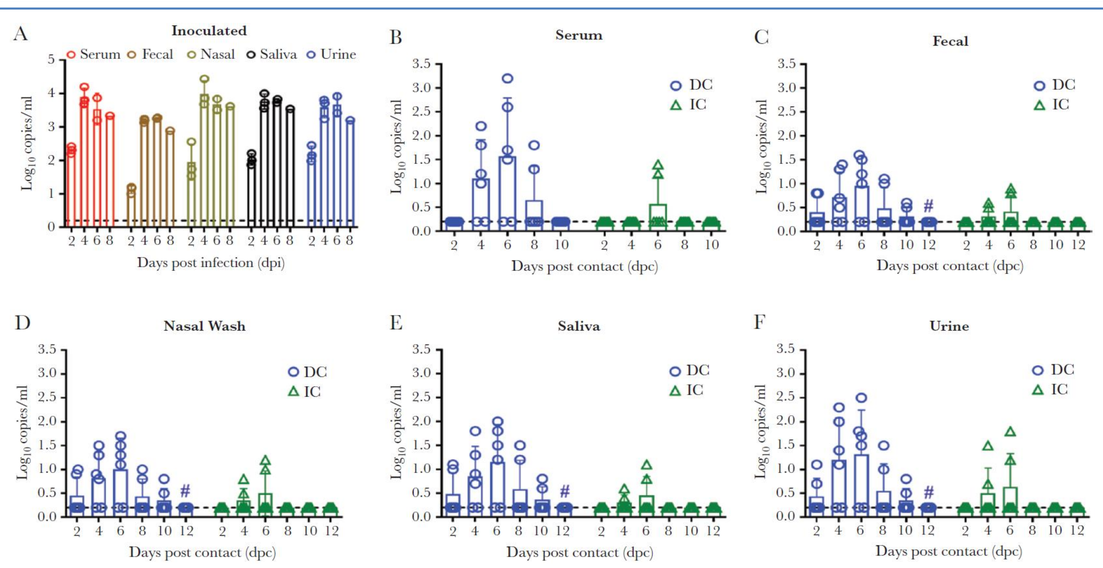


Figure 2. Viral titers in collected specimens from each group of ferrets. Specimens (fecal, nasal washes, saliva, and urine) were collected for 12 days, and blood samples were collected for 10 days (once every 2 days). All specimens from directly infected ferrets (A), and the serum (B), fecal (C), nasal washes (D), saliva (E), and urine (F) of direct contact (DC) and indirect contact (IC) ferrets were titered using real-time polymerase chain reaction. Data are presented with the horizontal dotted line as the minimum values (0.2 log_{10} copies/mL) observed.

Group	Organs of Deceased Ferrets, Log_{10} Copies/mL[a]				
	Lung	Liver	Spleen	Intestine	Kidney
Infected	4.02 ± 0.13	4.36 ± 0.58	5.28 ± 0.20	4.68 ± 0.45	3.04 ± 0.64
Fatal cases in DC	1.19 ± 0.08	2.63 ± 0.29	3.59 ± 0.18	1.14 ± 0.25	0.70 ± 0.15

Abbreviations: DC, direct contact; RT-PCR, reverse transcription polymerase chain reaction.
[a]The virus RNA detection limit was 0.30 log_{10} copies/mL.

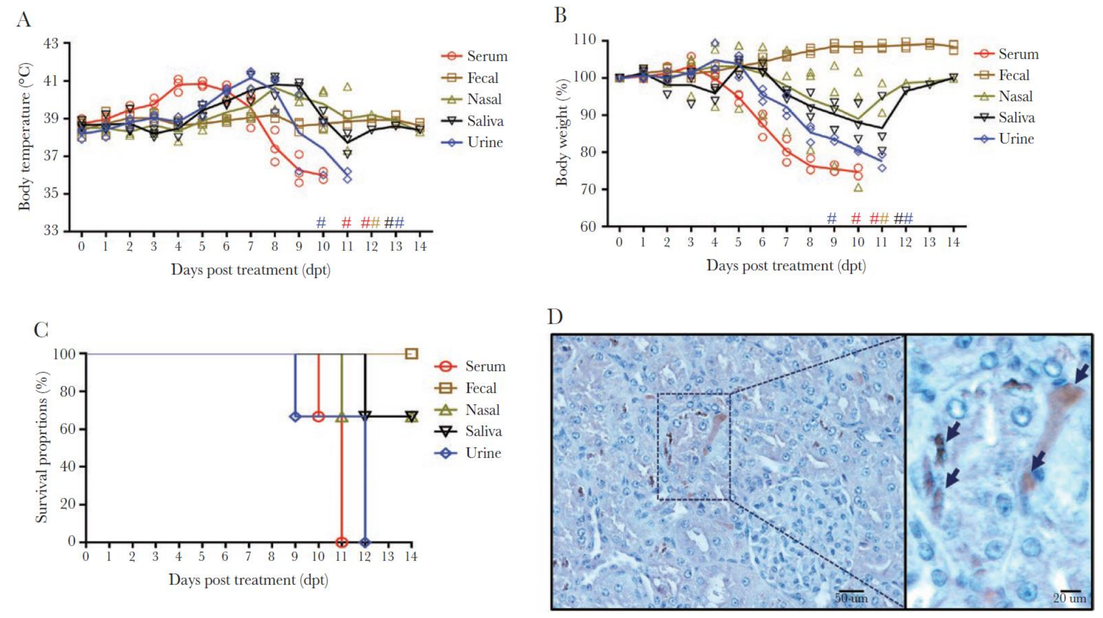


Figure 3. Clinical symptoms in ferrets (n = 3) after inoculation with collected body secretions (serum, fecal, nasal washes, saliva, or urine) by the oro-nasal route. Body temperature (A), relative weight (B), and survival rate (C) were assessed and are shown as means with standard deviations. The number indicates a reduced number of samples collected due to death of ferrets in this group.

Route	Specimen	Days Post-treatment, Log10 Copies/mLa					
		2	4	6	8	10	12
Serum	Serum	-	-	0.92 ± 0.38	1.59 ± 0.40	-	-
	Fecal	-	-	-	-	-	-
	Nasal	-	1.16 ± 0.34	1.77 ± 0.49	2.28 ± 0.40	-	-
	Saliva	-	0.72 ± 0.30	1.31 ± 0.57	1.12 ± 0.52	-	-
	Urine	-	1.04 ± 0.33	1.93 ± 0.39	2.15 ± 0.39	-	-
Fecal	Serum	-	0.33 ± 0.58	0.35 ± 0.60	-	-	-
	Fecal	-	-	-	-	-	-
	Nasal	-	-	-	-	-	-
	Saliva	-	-	-	-	-	-
	Urine	-	-	-	-	-	-
Nasal	Serum	-	0.49 ± 0.85	1.03 ± 0.91	1.45 ± 1.27	-	-
	Fecal	-	-	-	-	-	-
	Nasal	-	0.85 ± 0.74	1.64 ± 0.66	1.99 ± 0.76	2.57 ± 0.82	0.47 ± 0.29
	Saliva	-	0.56 ± 0.65	0.94 ± 0.88	0.54 ± 0.93	-	-
	Urine	-	1.02 ± 0.25	1.45 ± 0.36	2.33 ± 0.56	-	-
Saliva	Serum	-	0.75 ± 0.77	1.32 ± 0.69	2.02 ± 0.50	-	-
	Fecal	-	0.63 ± 0.75	0.95 ± 0.83	1.28 ± 1.11	1.11 ± 0.97	-
	Nasal	-	1.41 ± 0.55	1.73 ± 0.54	2.48 ± 1.64	2.53 ± 2.19	-
	Saliva	-	-	0.87 ± 0.86	1.68 ± 0.57	0.67 ± 1.16	0.37 ± 0.47
	Urine	-	-	1.19 ± 0.34	1.94 ± 0.44	3.01 ± 1.16	0.30 ± 0.53
Urine	Serum	-	0.47 ± 0.82	1.18 ± 1.06	1.91 ± 1.76	-	-
	Fecal	0.48 ± 0.84	1.39 ± 0.17	2.09 ± 0.41	2.82 ± 0.91	1.21 ± 0.69	-
	Nasal	-	0.49 ± 0.64	1.15 ± 1.00	1.94 ± 1.70	0.43 ± 0.91	-
	Saliva	0.37 ± 0.64	0.99 ± 0.33	1.50 ± 0.23	2.28 ± 0.55	0.30 ± 0.59	
	Urine		0.80 ± 0.94	1.82 ± 0.52	2.61 ± 0.55	1.24 ± 0.42	0.35 ± 0.60

[a]The virus RNA detection limit was 0.30 log_{10} copies/mL.

Discussion

1. **The SFTS clinical symptoms were observed from SFTSV infected ferrets and direct contacted (DC) ferrets such as high fever, weight loss and severe thrombocytopenia.**
2. **The SFTSV RNA was detected from various specimens (serum, fecal, nasal wash, saliva and urine) of ferrets and tissues (lung, liver, spleen, intestine and kidney) of deceased ferrets even in DC group.**
3. **The qRT-PCR results demonstrate that SFTSV can replicate in multiple organs, causing systemic infection, and be transmitted to direct contact naïve ferrets.**
4. **Most of body secretions (serum, fecal, nasal wash, saliva and urine) from SFTSV infected ferrets contained infectious viruses which a high enough to induce fatal infections.**
5. **SFTSV infected ferrets shed the virus more than 12 days from the direct and indirect SFTSV infections.**
6. **The patient's urine specimens could be a source for SFTSV for nosocomial infection.**
7. **Taken together, these results demonstrate that SFTSV can be shed in various body fluids of infected hosts for more than 12 days and that these specimens could be a source for direct and indirect SFTSV transmission to those in close contact, including other patients or health care professionals.**

Reference

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