

Risk assessment for safe handling of severe fever with thrombocytopenia virus.

A novel phlebovirus (1) causing severe human disease in China was isolated in 2011, and is referred to by different groups as severe fever with thrombocytopenia syndrome virus (SFTSV) (2), Huaiyangshan virus (3) or Henan fever virus (4). The name SFTSV will be used in this risk assessment in which the virus is proposed as belonging to Hazard Group 3 (HG3) and hence to be handled under Category 3 Containment conditions.

Background

The *Phlebovirus* genus (one of five genera in the family *Bunyaviridae*) currently comprises ten species (5) (representing approx 70 viruses) that can be divided into two groups, the phlebotomus (or sandfly) fever group (which includes Rift Valley fever virus (RVFV), Toscana virus (TOSV) and sandfly fever Sicilian virus), and the Uukuniemi group (which includes Uukuniemi virus). The S segment of phleboviruses has an ambisense coding strategy, with the N protein encoded in the negative-sense and the non-structural NSs protein encoded in the positive-sense of the genomic RNA; the M and L segments are conventional negative-sense RNAs. There are important differences between the two groups of phleboviruses. The phlebotomus fever group viruses have a sequence for a second nonstructural protein, NSm, encoded at the N-terminus of the glycoprotein precursor upstream of Gn and Gc, whereas the Uukuniemi group viruses just encode Gn and Gc proteins. The phlebotomus fever group viruses are transmitted by sandflies, mosquitoes or culicoides midges while Uukuniemi group viruses are transmitted by ticks. Finally, whereas phlebotomus fever group viruses include several human pathogens, the Uukuniemi group viruses are not associated with human disease (6).

SFTSV was isolated from patients presenting with fever, thrombocytopenia, leukocytopenia, and, in a few cases, multiple organ failure (2). To date about 500 cases have been confirmed in China with an overall fatality of about 12%.

Phylogenetic analysis of SFTSV genome sequences showed the virus to represent a third group in the *Phlebovirus* genus, nearly equidistant from the phlebotomus fever and Uukuniemi groups (2, 3). Its M segment does not encode an NSm equivalent protein. Further analysis of available genome sequences by Magiorkinis indicates that “SFTSV was probably introduced in these provinces in 2007 and was not circulating in this specific region before 1975” (7). Thus the virus can be regarded as “truly emerging” (1).

SFTSV has not been categorized by the Advisory Committee on Dangerous Pathogens (ACDP). Only one publication (4) addresses biosafety and states that SFTSV was handled under Category 3 Containment. To our knowledge the virus has not been made available to researchers outside of China and hence no other categorisation of the virus is available.

The current ACDP (8) classification of *Bunyaviridae* is shown below:

BUNYAVIRIDAE		
Akabane	3	
Bunyamwera	2	
California encephalitis	2	
Germiston	3	
Oropouche	3	
Hantaviruses:		
Belgrade (Dobrava)	3	
Hantaan (Korean haemorrhagic fever)	3	
Prospect Hill	2	
Puumala	2	
Seoul	3	
Sin Nombre (formerly Muerto Canyon)	3	
Other Hantaviruses	2	
Nairoviruses:		
Bhanja	3	
Crimean/Congo haemorrhagic fever	4	
Hazara	2	
Phleboviruses:		
Rift valley fever	3	V
Sandfly fever	2	
Toscana	2	
Other Bunyaviridae known to be pathogenic	2	

Viruses highlighted by shading are those currently studied in our laboratory and for which we have HSE approval to work with. Note that although Rift Valley fever virus is marked with V, indicating a vaccine is available, no vaccine is licenced for human use.

Assessing the risks

1: Identification of the hazards

Pathogenicity

SFTS is characterised by high fever ($>38^{\circ}\text{C}$), thrombocytopenia ($2 \times 10^{10} - 6 \times 10^{10}/\text{L}$ or lower in severe cases), leukocytopenia ($1 \times 10^9 - 3 \times 10^9/\text{L}$ or lower in severe cases), and gastrointestinal symptoms. In a small number of patients, SFTS progresses rapidly to multiorgan failure, which is fatal in some cases. Haemorrhagic manifestations have been observed in a few cases. According to Zhang et al. (10) “a fatal outcome was associated with high viral RNA load in blood at admission, as well as higher serum liver transaminase levels, more pronounced coagulation disturbances (activated partial thromboplastin time, thrombin time), and higher levels of acute phase proteins (phospholipase A, fibrinogen, hepcidin), cytokines (interleukin [IL]-6, IL-10, interferon- γ), and chemokines (IL-8, monocyte chemotactic protein 1, macrophage inflammatory protein 1b)”. Hence it was concluded that “viral replication and host immune responses play an important role in determining the

severity and clinical outcome in patients with infection by SFTSV”.

Epidemiology

SFTS disease has been detected in several provinces in China, and about 500 human cases have been described so far. In one study 3.6% (9/250) seropositivity in human sera in an endemic area was reported (11). Evidence suggests that SFTSV is a tick-borne virus. In the paper by Xu et al. (4) it is stated that “many (patients) had reported tick bites 7–9 days before illness”. SFTSV RNA has been detected in the ixodid ticks *Haemaphysalis longicornis* (2, 3) and to a lesser extent *Rhipicephalus* (formerly *Boophilus*) *microplus* (3), and the virus has been isolated from *H. longicornis* (2). *H. longicornis* has a distribution throughout China, Korea, Japan, Australia and New Zealand (12), while *R. microplus* has a more global distribution in subtropical and tropical regions of Asia, north-eastern Australia, Madagascar, south-eastern Africa, the Caribbean, South and Central America and Mexico (13). Thus SFTSV could be more widespread than the 7 provinces in China where it has been detected so far, and indeed a possible case imported from North Korea into Dubai has been described (14).

The mammalian reservoir has yet to be identified, though goats, cattle and hedgehogs are possibilities as they show high seroprevalence (11). Two papers report person-to-person transmission of SFTSV but in both papers transmission is associated with direct contact with the blood of infected patients (9, 15); no evidence for aerosol transmission has been reported.

Infectious dose

No information is available, but the infectious dose is likely to be small as it is tick-transmitted.

Routes of transmission

The natural route of transmission appears to be tick bite and perhaps exposure to infected human blood. Potential routes of exposure in the laboratory would be by penetrating sharps injury.

Medical data

No vaccines or therapeutic interventions are available. No information is available in the literature concerning supportive treatment given to hospitalised patients who recovered. The clinical illness caused by SFTSV is characterized by nonspecific symptoms and signs, including high fever, severe malaise, nausea, vomiting, and diarrhea, with manifest bleeding tendencies in some patients. Laboratory abnormalities share several features with other viral hemorrhagic fevers, such as leukopenia, severe thrombocytopenia, and coagulation abnormalities. Therefore, SFTS needs to be differentiated from human anaplasmosis, haemorrhagic fever with renal syndrome, and leptospirosis (2).

Environmental stability of agent

Studies on different bunyaviruses show that they are relatively stable in culture medium, depending on the temperature, but are rapidly inactivated in dry conditions (no infectivity detected after 24 hours at room temperature), and they are rapidly inactivated by exposure to 70% ethanol (16). In addition, Virkon at 1% has been validated to be effective against bunyaviruses and will be used as a disinfectant.

2: Consideration of the nature of the work

All work will be cell culture based; no work in animals will be carried out. All the work will be small-scale: virus grow-ups will generate up to 100ml of stocks (4 large flasks, 25ml medium per flask), while other experiments usually involve small flasks with 5ml of medium.

The research will be based on the establishment of a reverse genetics system to recover infectious virus from cloned cDNA copies of the viral genome, as detailed in *GM Project GM317/08.1: Molecular biology of hazard group 3 bunyaviruses*. Initially, wild type virus will be recovered from cDNA clones provided by our collaborators in China. Once the system has been established, research will entail modification of the cDNA clones to create viruses carrying defined mutations in the genome. These experiments will be similar to those outlined in GM317 for other HG3 bunyaviruses, and include mutagenesis of virus proteins and noncoding sequences of virus genome segments, rearrangements of the virus genome, and expression of foreign marker genes such as luciferase or green fluorescent protein. Based on our current experience with Rift Valley fever phlebovirus, the types of modification that will be introduced result in attenuation of virus replication. No modifications that will be introduced would result in the virus being more hazardous than the wild type.

All work will be carried out in the Category 3 Containment laboratory according to our existing and approved Code of Practice. All work with infectious material will be done in a Class II microbiological safety cabinet (MSC).

3: Evaluation the risks and selection of control measures

Infection by SFTSV naturally occurs by tick bite or exposure to infected patient blood. The risk of infection to laboratory workers will be reduced as (a) no sharps are permitted in the Containment Level 3 laboratory, and (b) all work with infectious material will be carried out in a Class II MSC. No additional personal protective equipment above that required by our existing CL3 Code of Practice is required to work with SFTSV.

Harvesting of infected cell culture supernatants (including centrifugation) and opening of vials could potentially produce aerosols. All handling of infected cells and supernatants will be performed in a Class II MSC to minimize the risk. Centrifugation will be carried out in sealed tubes in sealed rotors that will be loaded and opened in a Class II MSC according the CL3 Laboratory standard operating procedures.

Virkon at 1% will be used as a disinfectant as it has been validated to effective against bunyaviruses. All material will be autoclaved before leaving the Containment Level 3 laboratory according the CL3 Laboratory standard operating procedures.

The arthropod host could only be infected by taking a blood meal from an appropriate viraemic small mammal; since no animal work is proposed there should be no transfer to tick vectors even if competent vectors are present in the UK.

Segmented genome viruses can reassort their genome segments following coinfection of the same cell. For bunyaviruses reassortment can only occur between closely related viruses. It does not occur between viruses in different genera. As the

phylogenetic evidence indicates that SFTSV is a distinct third group in the *Phlebovirus* genus, it is not expected that it could reassort with members of the Uukuniemi or phlebotomus fever groups (e.g. Rift Valley fever virus). No additional risk is anticipated should reassortment occur between a modified, recombinant SFTSV and wild type SFTSV as the properties of the reassortant virus would be the same as the parental virus.

4. Proposed categorization of SFTSV

The following points are relevant to the proposed categorisation of SFTSV in comparison with other bunyaviruses. (i) Rift Valley fever virus is a mosquito-transmitted phlebovirus that can also be transmitted by the aerosol route. RVFV can cause severe human disease, including haemorrhagic fever, with an overall 1% mortality. Although veterinary vaccines against RVFV are available to vaccine is currently licenced for human use. Rift Valley fever virus is classified as HG3. (ii) Hantaan virus is a rodent-borne virus that is transmitted to humans via the aerosol route. It causes haemorrhagic fever with an overall 15% mortality. No vaccine is available. It is classified as HG3.

Hence we do not consider the risks of handling SFTSV to be higher than those of working with Rift Valley fever virus or Hantaan virus, and therefore we propose that SFTSV be similarly classified as a Hazard Group 3 virus.

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Richard M. Elliott

20/1/2012SFTSV Risk Assessment supporting Information

Reference details from Richard Elliott's document

1. Feldmann H. 2011. *N Engl J Med* 364: 1561-3
2. Yu et al. 2011. *N Engl J Med* 364: 1523-32
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Ref 3

Yong-Zhen Zhang, Dun-Jin Zhou, Xin-Cheng Qin, Jun-Hua Tian, Yanwen Xiong, et al. The ecology, genetic diversity and phylogeny of Huaiyangshan virus in China First published December 2011, doi: 10.1128/ Journal Virology JVI.06192-11

ABSTRACT

Surveys were carried out to better understand the tick vector ecology and genetic diversity of Huaiyangshan virus (HYSV) in both endemic and non-endemic regions. *Haemaphysalis longicornis* ticks were dominant in endemic regions, while *Rhipicephalus microplus* is more abundant in non-endemic regions. HYSV RNA was found in human and both tick species with more prevalence in *H. longicornis* and lesser in *R. microplus*. Phylogenetic analyses indicate that HYSV is a novel species of the genus *Phlebovirus*.

Ref 5

Gustavo Palacios, Amelia Travassos da Rosa, Nazir Savji, Wilson Sze, Ivan Wick, et al *Aguacate virus*, a new antigenic complex of the genus *Phlebovirus* (family *Bunyaviridae*) *J Gen Virol* June 2011 vol. 92 no. 6 1445-1453

ABSTRACT

Genomic and antigenic characterization of Aguacate virus, a tentative species of the genus *Phlebovirus*, and three other unclassified viruses, Armero virus, Durania virus and Ixcanal virus, demonstrate a close relationship to one another. They are distinct from the other nine recognized species within the genus *Phlebovirus*. We propose to designate them as a new (tenth) serogroup or species (*Aguacate virus*) within the genus. The four viruses were all isolated from phlebotomine sandflies (*Lutzomyia* sp.) collected in Central and South America. Aguacate virus appears to be a natural reassortant and serves as one more example of the high frequency of reassortment in this genus.

Ref 6

BUNYAVIRIDAE: MOLECULAR AND CELLULAR BIOLOGY | BOOK

Publisher: Caister Academic Press

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Publication date: September 2011

ISBN: 978-1-904455-90-5

Price: GB £159 or US \$310 (hardback)

Pages: viii + 214

Ref 10.

Yong-Zhen Zhang, Yong-Wen He, Yong-An Dai, Yanwen Xiong, Han Zheng, Dun-Jin Zhou et al Hemorrhagic Fever Caused by a Novel Bunyavirus in China: Pathogenesis and Correlates of Fatal Outcome Clin Infect Dis. (2011) doi: 10.1093/cid/cir804

Abstract

Background. Hemorrhagic fever-like illness caused by a novel Bunyavirus, Huaiyangshan virus (HYSV, also known as Severe Fever with Thrombocytopenia virus [SFTSV] and Fever, Thrombocytopenia and Leukopenia Syndrome [FTLS]), has recently been described in China.

Methods. Patients with laboratory-confirmed HYSV infection who were admitted to Union Hospital or Zhongnan Hospital between April 2010 and October 2010 were included in this study. Clinical and routine laboratory data were collected and blood, throat swab, urine, or feces were obtained when possible. Viral RNA was quantified by real-time reverse-transcriptase polymerase chain reaction. Blood levels of a range of cytokines, chemokines, and acute phase proteins were assayed.

Results. A total of 49 patients with hemorrhagic fever caused by HYSV were included; 8 (16.3%) patients died. A fatal outcome was associated with high viral RNA load in blood at admission, as well as higher serum liver transaminase levels, more pronounced coagulation disturbances (activated partial thromboplastin time, thrombin time), and higher levels of acute phase proteins (phospholipase A, fibrinogen, hepcidin), cytokines (interleukin [IL]-6, IL-10, interferon- γ), and

chemokines (IL-8, monocyte chemotactic protein 1, macrophage inflammatory protein 1b). The levels of these host parameters correlated with viral RNA levels. Blood viral RNA levels gradually declined over 3–4 weeks after illness onset, accompanied by resolution of symptoms and laboratory abnormalities. Viral RNA was also detectable in throat, urine, and fecal specimens of a substantial proportion of patients, including all fatal cases assayed.

Conclusions. Viral replication and host immune responses play an important role in determining the severity and clinical outcome in patients with infection by HYSV.

Ref 11

Yongjun Jiao, Xiaoyan Zeng, Xiling Guo, Xian Qi, et al. Preparation and evaluation of recombinant severe fever with thrombocytopenia syndrome virus nucleocapsid protein for detection of total antibodies in human and animal sera by double-antigen sandwich enzyme-linked immunosorbent assay. *J. Clin. Microbiol. February 2012 vol. 50 no. 2 372-377*

ABSTRACT

The recent emergence of the human infection confirmed to be caused by severe fever with thrombocytopenia syndrome virus (SFTSV) in China is of global concern. Safe diagnostic immunoreagents for determination of human and animal seroprevalence in epidemiological investigations are urgently needed. This paper describes the cloning and expression of the nucleocapsid (N) protein of SFTSV. An N-protein-based double-antigen sandwich enzyme-linked immunosorbent assay (ELISA) system was set up to detect the total antibodies in human and animal sera. We reasoned that as the double-antigen sandwich ELISA detected total antibodies with a higher sensitivity than traditional indirect ELISA, it could be used to detect SFTSV-specific antibodies from different animal species. The serum neutralization test was used to validate the performance of this ELISA system. All human and animal sera that tested positive in the neutralization test were also positive in the sandwich ELISA, and there was a high correlation between serum neutralizing titers and ELISA readings. Cross-reactivity was evaluated, and the system was found to be highly specific to SFTSV; all hantavirus- and dengue virus-confirmed patient samples were negative. SFTSV-confirmed human and animal sera from both Anhui and Hubei Provinces in China reacted with N protein in this ELISA, suggesting no major antigenic variation between geographically disparate virus isolates and the suitability of this assay in nationwide application. ELISA results showed that 3.6% of the human serum samples and 47.7% of the animal field serum samples were positive for SFTSV antibodies, indicating that SFTSV has circulated widely in China. This assay, which is simple to operate, poses no biohazard risk, does not require sophisticated equipment, and can be used in disease surveillance programs, particularly in the screening of large numbers of samples from various animal species.

Ref 12

Tenquist JD, Charleston WAG. A revision of the annotated checklist of ectoparasites of terrestrial mammals in New Zealand. *J R Soc N Z* 2001;31:481-542.

Ref 15

Chang-jun Bao, Xi-ling Guo, Xian Qi, Jian-li Hu, Ming-hao Zhou, Jay K. Varma et al, A Family Cluster of Infections by a Newly Recognized Bunyavirus in Eastern China, 2007: Further Evidence of Person-to-Person Transmission *Clin Infect Dis.* (2011) 53 (12): 1208-1214.

Abstract

Background. Seven persons in one family living in eastern China developed fever and thrombocytopenia during May 2007, but the initial investigation failed to identify an infectious etiology. In December 2009, a novel bunyavirus (designated severe fever with thrombocytopenia syndrome bunyavirus [SFTSV]) was identified as the cause of illness in patients with similar clinical manifestations in China. We reexamined this family cluster for SFTSV infection.

Methods. We analyzed epidemiological and clinical data for the index patient and 6 secondary patients. We tested stored blood specimens from the 6 secondary patients using real time reverse transcription polymerase chain reaction (RT-PCR), viral culture, genetic sequencing, micro-neutralization assay (MNA), and indirect immunofluorescence assay (IFA).

Results. An 80-year-old woman with fever, leucopenia, and thrombocytopenia died on 27 April 2007. Between 3 and 7 May 2007, another 6 patients from her family were admitted to a local county hospital with fever and other similar symptoms. Serum specimens collected in 2007 from these 6 patients were positive for SFTS viral RNA through RT-PCR and for antibody to SFTSV through MNA and IFA. SFTSV was isolated from 1 preserved serum specimen. The only shared characteristic between secondary patients was personal contact with the index patient; none reported exposure to suspected animals or vectors.

Conclusions. Clinical and laboratory evidence confirmed that the patients of fever and thrombocytopenia occurring in a family cluster in eastern China in 2007 were caused by a newly recognized bunyavirus, SFTSV. Epidemiological investigation strongly suggests that infection of secondary patients was transmitted to family members by personal contact.

Additional Ref

Liu Y, Li Q, Hu W, Wu J, Wang Y, Mei L, Walker DH, Ren J, Wang Y, Yu XJ. Person-to-person transmission of severe fever with thrombocytopenia syndrome virus. *Vector Borne Zoonotic Dis.* 2011 Sep 28. [Epub ahead of print]

Abstract

Abstract Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by a newly discovered bunyavirus, SFTS virus (SFTSV), and causes high fatality (12% on average and as high as 30%). The objective of this study was to determine whether SFTSV could be transmitted from person to person. We analyzed sera of 13 patients from two clusters of unknown infectious diseases that occurred between September and November of 2006 in Anhui Province of China for SFTSV antibody by indirect immunofluorescence assay and for SFTSV RNA by

RT-PCR. We found that all patients (n=14) had typical clinical symptoms of SFTS including fever, thrombocytopenia, and leukopenia and all secondary patients in both clusters got sick at 6-13 days after contacting or exposing to blood of index patients. We demonstrated that all patients in cluster 1 including the index patient and nine secondary patients and all three secondary patients in cluster 2 had seroconversion or fourfold increases in antibody titer to SFTSV and/or by RT-PCR amplification of SFTSV RNA from the acute serum. The index patient in cluster 2 was not analyzed because of lack of serum. No person who contacted the index patient during the same period, but were not exposed to the index patient blood, had got illness. We concluded that SFTSV can be transmitted from person to person through contacting patient's blood.