### Bunyavirus

<table>
<thead>
<tr>
<th>Family</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Flavivirus</td>
<td>Dengue, yellow fever &amp; TBE</td>
</tr>
<tr>
<td>Arenavirus</td>
<td>Lassa, Junin, Machupo, Guanarito</td>
</tr>
<tr>
<td>Filovirus</td>
<td>Ebola, Marburg</td>
</tr>
<tr>
<td>Bunyavirus</td>
<td>CCHF, RVF, Hantaviruses</td>
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</tbody>
</table>

**Viral haemorrhagic fever**

Enveloped RNA Virus
Family *Bunyaviridae* contains the genera

- Genus *Hantavirus*: Hantaan virus
- Genus *Nairovirus*: CCHF, Dugbe virus
- Genus *Orthobunyavirus*: Bunyamwera virus
- Genus *Phlebovirus*: Rift Valley fever virus
- Genus *Tospovirus*: Tomato spotted wilt virus

Detected in 1940s, named in 1975
Transmitted by arthropod and rodents
Spherical virions with diameter of 80-120nm
Enveloped, with helical nucleocapsid, contains no matrix protein

Classification:
Morphology
Antigenicity
Genetic characteristics
Orthobunyavirus
Bunyamwera
Hantaan

Nairovirus
Dugbe

Phlebovirus
 Rift Valley fever

Tospovirus
Tomato spotted wilt

<table>
<thead>
<tr>
<th>Genus/Virus</th>
<th>Consensus S, M, L terminal nucleotides</th>
<th>Gene sizes (accession no.)</th>
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<tbody>
<tr>
<td>Orthobunyavirus</td>
<td>3' UCAUCACAUG-&lt;br&gt;5' AGUAGUGUGC-</td>
<td>S 961 M 4459 L 8275 (D100353) (M11852) (X14365)</td>
</tr>
<tr>
<td>Hantaan</td>
<td>3' UCAUCACAUG-&lt;br&gt;5' AGUAGUGUGC-</td>
<td>S 1696 M 3616 L 8533 (M14629) (M14627) (X55901)</td>
</tr>
<tr>
<td>Dugbe</td>
<td>3' AGAGUUCU-&lt;br&gt;5' UUCAGAAGAA-</td>
<td>S 1712 M 4888 L 12236 (M25150) (M94133) (L150216)</td>
</tr>
<tr>
<td>Rift Valley fever</td>
<td>3' UCGUCAU-&lt;br&gt;5' AGAGAAG-</td>
<td>S 1090 M 3885 L 8444 (X53771) (M1157) (X56464)</td>
</tr>
<tr>
<td>Tomato spotted wilt</td>
<td>3' UCGUUCCU-&lt;br&gt;5' AGAGCAAU-</td>
<td>S 2915 M 4921 L 8997 (D10645) (S48091) (D100366)</td>
</tr>
</tbody>
</table>

**Genome Have Same Coding Strategy**

<table>
<thead>
<tr>
<th>Genus/RNA</th>
<th>S segment ORFs and coding direction</th>
<th>Protein (size)</th>
<th>Coding Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthobunyavirus/cRNA</td>
<td>500</td>
<td>N (19-25 kD) NSp (10-13 kD)</td>
<td>negative sense</td>
</tr>
<tr>
<td>Hantavirus/cRNA</td>
<td>500 1000 1500</td>
<td>N (50-54 kD)</td>
<td>negative sense</td>
</tr>
<tr>
<td>Nairovirus/cRNA</td>
<td>500 1000 1500</td>
<td>N (48-54 kD)</td>
<td>negative sense</td>
</tr>
<tr>
<td>Phlebovirus/cRNA</td>
<td>500 1000 1500</td>
<td>N (24-30 kD) NSp (29-31 kD)</td>
<td>ambisense</td>
</tr>
<tr>
<td>Tospovirus/cRNA</td>
<td>500 1000 1500 2000 2500</td>
<td>N (29 kD) NSp (32 kD)</td>
<td>ambisense</td>
</tr>
</tbody>
</table>
Orthobunyavirus
- cRNA
- G2 (29-41 kD)
- NS$_p$ (15-18 kD)
- G1 (106-120 kD)
- coding direction

Hantavirus
- cRNA
- G1 (68-76 kD)
- G2 (52-58 kD)
- coding direction

Nairovirus
- cRNA
- G2 (30-45 kD)
- G1 precursor (65 kD)
- G1 (72-84 kD)
- coding direction

Phlebovirus
- cRNA
- NS$_p$ (none-30 kD)
- G1/G2 (50-75 kD)
- G2/G1 (50-75 kD)
- coding direction

Tospovirus
- cRNA
- vRNA
- G2 (52-58 kD)
- G1 (75 kD)
- NS$_p$ (34 kD)
- coding direction

Orthobunyavirus
- cRNA
- L (259 kD)
- negative sense

Hantavirus
- cRNA
- L (246 kD)
- negative sense

Nairovirus
- cRNA
- L (459 kD)
- negative sense

Phlebovirus
- cRNA
- L (237 kD)
- negative sense

Tospovirus
- cRNA
- L (331 kD)
- negative sense
Coding proteins are significantly conservative

More than 30 viral types have been detected, among which more than 20 types could be causative factors for human infections.

Host animals can be classified in 3 different groups:
- *Sigmodontinae*: 新世界小鼠和大鼠
- *Murinae*: 旧世界小鼠和大鼠
- *Arvicolinae*: 田鼠和旅鼠
### Types of Hantavirus

<table>
<thead>
<tr>
<th>Virus</th>
<th>Epidemic Regions</th>
<th>Disease</th>
<th>Main hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hantaan virus</td>
<td>Asia, Europe</td>
<td>HFRS (EHF)</td>
<td>Apodemus agrarius</td>
</tr>
<tr>
<td>Seoul</td>
<td>Worldwide</td>
<td>HFRS (EHF)</td>
<td>Rattus norvegicus</td>
</tr>
<tr>
<td>Puumala</td>
<td>Europe, Asia</td>
<td>HFRS (NE)</td>
<td>Clethrionomys glareolus</td>
</tr>
<tr>
<td>Dobrava</td>
<td>Europe</td>
<td>HFRS</td>
<td>Apodemus flavicollis</td>
</tr>
<tr>
<td>Prospect Hill</td>
<td>North America, Russia</td>
<td>Not pathogenic</td>
<td>Microtus pennsylvanicus</td>
</tr>
<tr>
<td>Thailand</td>
<td>Thailand</td>
<td>Unknown</td>
<td>Bandicota indica</td>
</tr>
<tr>
<td>Thottapalayam</td>
<td>India</td>
<td>Unknown</td>
<td>Suncus murinus</td>
</tr>
<tr>
<td>Tula</td>
<td>Europe</td>
<td>HFRS</td>
<td>Microtus arvalis</td>
</tr>
<tr>
<td>Khabarovask</td>
<td>Russia</td>
<td>Unknown</td>
<td>Microtus fortis</td>
</tr>
<tr>
<td>Sin Nombre</td>
<td>North America</td>
<td>HPS</td>
<td>Peromyscus maniculatus</td>
</tr>
<tr>
<td>Black Creek Canal</td>
<td>North America</td>
<td>HPS</td>
<td>Sigmodon hispidus</td>
</tr>
<tr>
<td>El Moro Canyay</td>
<td>North America</td>
<td>HPS</td>
<td>Reithrodontomys megalotis</td>
</tr>
<tr>
<td>Bayou</td>
<td>North America</td>
<td>HPS</td>
<td>Oryzomys palustris</td>
</tr>
</tbody>
</table>
Diseases caused by Hantavirus

1. Haemorrhagic Fever with Renal Syndrome (HFRS)
   Hantaan, Seoul (SOE), Puumala and Dobrava (DOB), etc.

2. Hantaviruses pulmonary syndrome (HPS)
   Sin Nombre (SNV), etc.

Hantavirus is harmless to its host. Host animals could carry it a lifetime.
Prevalence of Hantavirus is affected by climate

- Research showed that in 1992/93, due to the significant increase of rainfall, HPS outbreak occurred in 1993.
- Increased rainfall – harvest – increased density of mice – mice migrate to human residential areas when food supplies depleted – increased chances of infections
Genus *Nairovirus*

Tick-borne viruses (rare reports of transmission via culicoides flies and mosquitoes)

7 species, 34 strains. Most CCHF, Hazara virus and Nairobi sheep disease (NSD) (includes: NSD and Dugbe virus)

Pathogenic viruses to human include CCHF, NSD and Dugbe.

Based on genetic analysis of genome evolution, CCHF can be classified into 3 groups: Group A contains two subgroups: Subgroup Africa and Subgroup Asia, which include viruses isolated in Pakistan, China, Iran, Russia and Madagascar; group B contains viruses isolated in southern and western Africa and Iran; Group C only has one virus isolated in Greece. Geographic distribution of different virus groups mainly depend on the geographic distribution of ticks.

# Birds remain healthy after infection of CCHFV in the lab. No viraemia and antibody response observed. CCHF virus could also been isolated from ticks on birds, but ostrich are excluded. A large number of ostriches were put down due to CCHFV infections in South Africa.

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**Modes of Transmission**

- Human to human
- Ticks to human
- Animal to human
- Animal to ticks
- Tick to tick
Infection

- Infective Dose: 1 – 10 viruses, case fatality rate could be as high as 40%

- Incubation period ranges from 2 to 9 days. Incubation period of tick-transmitted disease could be as short as 1-3 days, which is shorter than the period of disease transmitted via animals (5-13 days). The normal incubation period for contact with infectious blood could be 5-6 days.

IgM could last for 2-3 months and up to 6 months...
Diagnosis

- Clinical presentations and history of contacts
- Differentiate diagnosis
- Virus isolation: suckling mice inoculated with samples. Cell culture and isolation, LLC-MK2, Vero, Vero E6, BHK-21, and SW-13. within 4-7 days, 10(7)-10(8) pfu could be cultured and it could be detected on the 5th day by IFA.
- Antibody detection: based on nucleoprotein ELISA, inactivated virus ELISA and IFA, Levels of IgM and IgG could be tested 7 days after onset of illness by ELISA and IFA. Level of IgM antibody would decrease to an undetectable level 4 months
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- Diagnostic testing: RT-PCR, real-time PCR
- Nested-RT-PCR
  - Forward: F2: 5'-TGG ACA CCT TCA CAA ACT A-3' [135-153]
  - Reverse: R2: 5'-GAC ATC ACA ATT TCA CCA GG-3' [549-530]
  - Product Size: 260 bp
- Nested primers: F3, R3
  - Forward: F3: 5'-GAA TGT GCA TGG GTT AGC TC-3' [290-309]
  - Reverse: R3: 5'-GAC AAA TTC CCT GCA CCA-3' [670-653]
  - Product Size: 220 bp
- Real-time PCR
  - CCHF L1, CCHF D1
    - Forward: CCHF L1: 5'-GCTTGGTCAGCCTCTACTGG-3' [nt position at the Drosdov strain: 294-313]
    - Reverse: CCHF D1: 5'-TGCTTGGACACGGAAACCTA-3' [nt: 463-482]
    - Real-time-probe: probe CCHF S1: 5' AGAAGGGCTTGAGTGGTT
Geographic Distribution of CCHFV

Symptoms and Signs

- Fever (39-41°C), lasts for 5-12 days, or exhibits biphasic fever. 81.8%
- 63.6% diarrhoea
- Symptoms of haemorrhagic fever, severe cases may develop symptoms of haemorrhagic fever 3-6 days after onset of disease. Petechiae and large subcutaneous ecchymosis may present mainly on the upper body and arms. Haemafecia, haematemesis and bleeding nose may present 4-5 days after onset of disease. Meanwhile, patients may present with genital tract or gum bleeding. Critical cases may present with cerebral haemorrhage.
- Vertigo
- 18.2% with sore throat
- 45.5% with vomiting
- Nausea
- Serious headache
- Muscle pain
- CNS symptoms, negative prognosis once CNS symptoms present
Massive cutaneous ecchymosis

Massive cutaneous ecchymosis on the arm of a CCHF patient, 7-10 days after clinical onset. Photograph courtesy of Dr. Robert Swanepoel, National Institute of Virology, South Africa (Whitehouse, 2004)

Orthobunyavirus

More than 150 species. Globally distributed

Mosquito-borne, amplifying in vertebrate hosts
First bunyamwera virus was isolated in 1940s in Uganda. Several sero-groups have been isolated so far:
  - California Serogroup Viruses: mainly in US
  - Bunyamwera Serogroup Viruses: mainly in sub-Sahara Africa and North America
  - Simbu Serogroup Viruses: mainly in South America, also detected in Asia-Pacific, middle-east and Africa

La Crosse virus
Clinical Characteristics

They cause similar encephalitis-like illness, except for infection with La Crosse. Children are prone to become serious cases. Adults are more affected by infection with Jamestown Canyon. Disease spectrum ranges from non-obvious symptoms, mild fever to deadly encephalitis.

The incubation period is 3-7 days. Main presentations include sudden onset of fever, with neck stiffness, sleepiness, headache, nausea and vomiting, etc. and patients recover within 7 days. About half patients may present with convulsion and 30% with coma and the course of disease is relatively long. 65% patients may have symptoms of meningitis and monocytes and PML could also be detected in CSF. Patients may present with mild neurological symptoms when they are discharged, but sequelae are rarely found. The most significant sequela is epilepsy. Around 10-15% children may have it and 2% adults may have permanent paralysis.

LaCrosse Viral Encephalitis

- Mainly infect children under age 16
- Aedes is the main media of transmission
- Sparrows and chipmunk are main host for virus amplification
- The most common bunyavirus infection in US
- Brain infection
- Symptoms include:
  - Fever
  - Convulsion
  - Sleepiness
  - Focal neurological signs
Phlebovirus

Phlebovirus is globally distributed, excluding Australia. Since most virus is related to phlebotomus, it is called phlebovirus. This virus is divided into two groups: Sandfly fever group and Uukuniemi. The former is mainly transmitted through phlebotomus and mosquitoes, while the latter is mainly through ticks.

The most important pathogen Rift Valley Fever virus (RVF) is mainly transmitted via aedes. RVFV was firstly isolated in 1930 from a lamb. It is widely spread among animals in sub-Saharan region. In 2000, it was firstly spread to Arabian Peninsula, out of Africa, and caused the first human and animal outbreak.

Sandfly fever virus Sicilian and Naples were firstly isolated in 1943 and 1944 respectively.

A: Rift valley fever virus (genus Phlebovirus)
B: Sin Nombre virus (genus Hantavirus)

(135,000x magnification). (Courtesy of Cynthia Goldsmith and Luanne Elliott).
**Rift Valley Fever**

- **Virulence factors**: negative-stranded RNA virus. Member of rift valley fever virus, genus *Phlebovirus*, family *Bunyaviridae*
- **Host**: sheep and ruminants
- **Media of transmission**: mosquitoes (Culex, Aedes, Anopheles, Erethmapodites, Mansonia, Culicoides, Coquillettidia spp.)
- **Mediator**: none
- **Incubation period**: 3d - 5d (range 2d - 7d)
- **Diagnostic methods**: virus isolation and culture (blood, hydrocephalus), serological tests; nucleic acid amplification, BSL3 lab.
- **Typical treatment measures**: supportive therapy, animal tests indicated that Ribavirin is relatively effective.
- **Vaccine**: commercially available
- **Clinical signs**: headache, muscle pain, sensitivity to light, joint pain, pimples, rarely jaundice, retinitis. Contact with lamb or camel is helpful for diagnosis and case fatality rate is 0.1%

**Modes of Transmission**

- **Mosquito to human**: mosquito bites
- **Animal to human**: exposure to excretes of infected animals (aerosol transmission), or contact with contaminated blood or meat
- **Mosquito to mosquito**: by eggs
Infection

• 1 to 10 viruses

• Infection of liver cells

• Incubation period: 2 to 6 days

• Symptoms and signs

Dynamic Changes of Virus and Antibodies in RVFV Infected Animals
RVFLaboratory Test

- Virus isolation: RVFV, 4 hours after infection of CV-1, Vero and BHK-2, the extracellular virus titer reaches at least 3.6 logs/ml; 22 hours after infection of CV-1, it reaches the peak titer: 7.7 logs/ml and 50% of cells developed lesions. Lesions in other cell lines need 45 hours. 22 hours after infection, RVF virus antigen could be detected in 3 cell lines by IFA, but the fluorescent focus in CV-1 is bigger with more positive cells. 22 hours after infection of CV-1, virions could be observed via electron microscopy, while it takes 45 hours in Vero and HBK-21. virions could be observed in liver tissue samples in autopsy by electron microscopy.
- Serological test
- Nucleic acid test

- RVF1 and RVF2
  - Forward: RVF1 777/5' GAC TAC CAG TCA GCT CAT TAC C 3'/798
  - Reverse: RVF2 1327/5' TG TGA ACA ATA GGC ATT GG 3'/1309
  - Product Size: 551 bp
- RVF3 and RVF4
  - Forward: RVF3 876/5' CAG ATG ACA GGT GCT AGC 3'/893
  - Reverse: RVF4 1249/5' CT ACC ATG TCC TCC AAT CTT GG 3'/1228
  - Product Size: 374 bp
Thank You!