

## CRIMEAN–CONGO HAEMORRHAGIC FEVER

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### SUMMARY

*Crimean–Congo haemorrhagic fever virus (CCHFV) of the genus Orthonairovirus of the family Nairoviridae causes a zoonotic disease in many countries of Asia, Africa, the Middle East and south-eastern Europe. As the distribution of CCHFV coincides with the distribution of its main vector, ticks of the genus Hyalomma, the spread of infected ticks into new, unaffected areas facilitates the spread of the virus. The virus circulates in a tick–vertebrate–tick cycle, but can also be transmitted horizontally and vertically within the tick population. Hyalomma ticks infest a wide spectrum of different wildlife species, e.g. deer and hares, and free-ranging livestock animals, e.g. goat, cattle, and sheep. Many birds are resistant to infection, but ostriches appear to be more susceptible. Viraemia in livestock is short-lived, and of low intensity. These animals play a crucial role in the life cycle of ticks, and in the transmission and amplification of the virus and are, therefore, in the focus of veterinary public health. As animals do not develop clinical signs, CCHFV infections have no effect on the economic burden regarding livestock animal production. In contrast to animals, infections of humans can result in the development of a severe disease, Crimean–Congo haemorrhagic fever (CHF).*

*Every year, more than 1000 human CHF cases are reported from Albania, Bulgaria, Kosovo and Turkey. In other countries, infection rates and case numbers are largely unknown. Case fatality rates of 5% (in Turkey) to 80% have been reported and may depend on the virus strain, education of the local population and the effectiveness of public health interventions. At present, the pathogenesis of the disease in humans is not well understood. Most people become infected by tick bites and by crushing infected ticks, but infection is also possible through contact with blood and other body fluids of viraemic animals. As CCHFV also has the potential to be transmitted directly from human-to-human, nosocomial outbreaks might occur.*

*There is no approved CHF vaccine available and therapy is restricted to treatment of the symptoms. Health education and information on prevention and behavioural measures are most important in order to enhance public risk perception and, therefore, decrease the probability of infections. Thus the identification of endemic areas is crucial for focused and targeted implementation of public health measures. Serological screening of ruminants allows CCHFV-affected areas to be identified, as antibody prevalence in animals is a good indicator of local virus circulation. Treatment with tick repellents can be quite effective in reducing the tick infestation of animals. To protect laboratory staff, handling of CCHFV infectious materials should only be carried out at an appropriate biocontainment level.*

**Identification of the agent:** *Only a single virus serotype is known to date although sequencing analysis indicates considerable genetic diversity. CCHFV has morphological and physicochemical properties typical of the family Nairoviridae. The virus has a single-stranded, negative-sense RNA genome consisting of three segments: L (large), M (medium) and S (small), each of which is contained in a separate nucleocapsid within the virion. The virus can be isolated from serum or plasma samples collected during the febrile or viraemic stage of infection, or from liver of infected animals. Primary isolations are made by inoculation of several tissue cultures, commonly African green monkey kidney (Vero) cells or by intracerebral inoculation of suckling mice. For identification and characterisation of the virus, conventional and real-time reverse transcriptase polymerase chain reaction (PCR) can be used. As infections of animals remain clinically inapparent, the likelihood of isolating virus from a viraemic animal is very low.*

**Serological tests:** Type-specific antibodies are demonstrable by indirect immunofluorescence test or by IgG-sandwich and IgM-capture ELISA. Currently there are no commercial test systems available for animal health; only a few in-house systems have been published or kits are used replacing the conjugate provided in kit with one that is suitable for the animal species to be screened for CCHFV-specific antibodies.

**Requirements for vaccines:** There is no vaccine available for animals.

## A. INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF) is a zoonotic disease caused by a tick-borne CCHF virus (CCHFV) of the genus *Orthonairovirus* of the family *Nairoviridae*, order *Bunyvirales*. CCHFV possesses a negative-sense RNA genome consisting of three segments, L (large), M (medium) and S (small) each contained in a separate nucleocapsid within the virion. All orthonairoviruses are believed to be transmitted by either ixodid or argasid ticks, and only three are known to be pathogenic to humans, namely CCHF, Dugbe and Nairobi sheep disease viruses (Swanepoel & Burt, 2004; Swanepoel & Paweska, 2011; Whitehouse, 2004). Recently CCHFV was grown successfully for the first time in several tick cell lines derived from both a natural vector (*Hyalomma anatolicum*) and other tick species not implicated in natural transmission of the virus (Bell-Sakyiet *al.*, 2012).

The virus from an outbreak of “Crimean haemorrhagic fever” in soldiers and peasants in the Crimean Peninsula in 1944 was not isolated or characterised until 1967. “Congo haemorrhagic fever” virus, isolated from a patient in the former Zaire (now Democratic Republic of the Congo) in 1956, was shown in 1969 to be the same virus. As a consequence the names of both countries have been used in combination to describe the disease (Hoogstraal, 1979). Distribution of the virus reflects the broad distribution of *Hyalomma* ticks, the predominant vector of the virus (Avsic-Zupanc, 2007; Grard *et al.*, 2011; Papa *et al.*, 2011; Swanepoel & Paweska, 2011).

The natural cycle of CCHFV includes transovarial and transstadial transmission among ticks and a tick-vertebrate-tick cycle involving a variety of wild and domestic animals. Infection can also be transferred between infected and uninfected ticks during co-feeding on a host; so called ‘non-viraemic transmission’ phenomenon. *Hyalomma* ticks feed on a variety of domestic ruminants (sheep, goats, and cattle), and wild herbivores, hares, hedgehogs, and certain rodents. CCHFV infection in animals was reviewed by Nalca *et al.*, (2007). Although animal infections are generally subclinical, the associated viraemia levels are sufficient to enable virus transmission to uninfected ticks (Swanepoel & Burt, 2004; Swanepoel & Paweska, 2011). Many birds are resistant to infection, but ostriches appear to be more susceptible than other bird species (Swanepoel *et al.*, 1998). Although they do not appear to become viraemic, ground feeding birds may act as a vehicle for spread of CCHFV infected ticks. Results from serological surveys conducted in Africa and Eurasia indicate extensive circulation of the virus in livestock and wild vertebrates (Swanepoel & Burt, 2004).

Humans acquire infection from tick bites, or from contact with infected blood or tissues from livestock or human patients. After incubation humans can develop a severe disease with a prehaemorrhagic phase, a haemorrhagic phase, and a convalescence period. Haemorrhagic manifestations can range from petechiae to large haematomas. Bleeding can be observed in the nose, gastrointestinal system, uterus and urinary tract, and the respiratory tract, with a mortality rate ranging from 5% to 80% (Ergonul, 2006; Yen *et al.*, 1985; Yilmaz *et al.*, 2008). The severity of CCHF in humans highlights the impact of this zoonotic disease on public health. Although CCHFV has no economic impact on livestock animal production, the serological screening of animal serum samples for CCHFV-specific antibodies is very important. As prevalence in animals is a good indicator for local virus circulation, such investigations allow identification of high-risk areas for human infection (Mertens *et al.*, 2013). Slaughterhouse workers, veterinarians, stockmen and others involved with the livestock industry should be made aware of the disease. They should take practical steps to limit or avoid exposure of naked skin to fresh blood and other animal tissues, and to avoid tick bites and handling ticks. Experiences from South Africa demonstrated that the use of repellents on animals before slaughter could reduce the numbers of infected slaughterhouse workers (Swanepoel *et al.*, 1998). The treatment of livestock in general can reduce the tick density among these animals and thus reduce the risk of tick bite in animal handlers (Mertens *et al.*, 2013). Such tick control by the use of acaricides is possible to some extent, but may be difficult to implement under extensive farming conditions. Inactivated mouse brain vaccine for the prevention of human infection has been used on a limited scale in Eastern Europe and the former USSR (Swanepoel & Paweska, 2011).

Infectivity of CCHFV is destroyed by boiling or autoclaving and low concentrations of formalin or beta-propiolactone. The virus is sensitive to lipid solvents. It is labile in infected tissues after death, presumably due to a fall in pH, but infectivity is retained for a few days at ambient temperature in serum, and for up to 3 weeks at 4°C. Infectivity is stable at temperatures below –60°C (Swanepoel & Paweska, 2011). CCHFV is classed in Risk Group 3 for human infection and should be handled with appropriate measures as described in Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*. Biocontainment measures should be determined by risk analysis as described in Chapter 1.1.4 (Palmer, 2011; Whitehouse, 2004).

## B. DIAGNOSTIC TECHNIQUES

**Table 1.** Diagnostic test formats for Crimean–Congo haemorrhagic fever virus infections in animals

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases in animals	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
<b>Identification of the agent<sup>1</sup></b>						
Real-time RT-PCR	–	+++	–	–	–	–
Virus isolation in cell culture	–	–	–	–	+	–
<b>Detection of immune response</b>						
IgG ELISA	+++	+	–	–	+++	–
Competitive ELISA	+++	+	–	–	+++	–
IgM ELISA	–	++	–	–	–	–

Key: +++ = recommended for this purpose; ++ recommended but has limitations;  
+ = suitable in very limited circumstances; – = not appropriate for this purpose.

ELISA = enzyme-linked immunosorbent assay; RT-PCR = reverse-transcription polymerase chain reaction.

CCHFV infection causes only a mild fever in domestic and wild vertebrate animals with a detectable viraemia of up to 2 weeks (Gonzalez *et al.*, 1998; Gunes *et al.*, 2011). Similarly infected ostriches develop only low and short-lived viraemia and no clinical signs (Swanepoel & Burt, 2004). Therefore, recent infections in animals are rarely diagnosed and methods such as polymerase chain reaction (PCR), virus isolation in cell culture and IgM detection by enzyme-linked immunosorbent assay (ELISA) are mainly used in human CCHF diagnostic or in the special case that an animal has to be classified as CCHFV free. For prevalence analysis and for determination of whether CCHFV is circulating in a country, methods for the detection of IgG antibodies are preferred (Table 1). If there is any possibility or suspicion that diagnostic samples could be contaminated with CCHFV, they should be handled under an adequate biosafety level and all persons dealing with those samples should be aware of the possible risk and should use personal protective equipment to avoid human infections.

### 1. Identification of the agent

For testing animals for viraemia, as well as in human clinical diagnosis, a rapid diagnosis can be achieved by detection of viral nucleic acid in serum or plasma using conventional (Burt *et al.*, 1998) or real-time reverse transcriptase (RT-) PCR (Drosten *et al.*, 2002; Duh *et al.*, 2006; Wölfel *et al.*, 2007), or by demonstration of viral antigen (Shepherd *et al.*, 1988). Specimens to be submitted for laboratory confirmation of CCHF include blood and liver samples. Because of the risk of laboratory-acquired infections, work with CCHFV should be conducted in appropriate biosafety facilities.

The virus can be isolated from serum and organ suspensions in a wide variety of cell cultures, including Vero, LLC-MK2, SW-13, CER and BHK21 cells, and identified by immunofluorescence using specific antibodies. Isolation and identification of virus can be achieved in 1–5 days, but cell cultures lack sensitivity and usually only detect high concentrations of virus present in the blood. Intracerebral inoculation of sucking mice is more sensitive than cell cultures for virus isolation, but is not recommended, on the grounds of animal welfare.

<sup>1</sup> A combination of agent identification methods applied on the same clinical sample is recommended.

## 1.1. Virus isolation in cell culture

CCHFV can be isolated in mammalian cell cultures. Vero cells are commonly used, usually yielding an isolate between 1 and 5 days post-inoculation (p.i). CCHFV is poorly cytopathic and thus infectivity is titrated by demonstration of immunofluorescence in infected cells (Shepherd *et al.*, 1986). SW-13 cell line has also been used extensively for virus isolation, producing plaques within 4 days (p.i.). Identification of a CCHFV isolate has to be confirmed by immunofluorescence or molecular techniques (Burt *et al.*, 1998; Shepherd *et al.*, 1986).

### 1.1.1. Test procedure

- i) Susceptible cell lines include Vero-E6, BHK-21, LLC-MK2 and SW-13 cells. Inoculate 80% confluent monolayers of the preferred cell line with the specimen. The volume of specimen to be used depends on the size of the culture vessel (i.e. 25 cm<sup>2</sup> culture flask or 6- or 24-well tissue culture plate). The specimen volume should be sufficient to cover the cell monolayer. Samples of insufficient volumes can be diluted with tissue culture medium to prepare sufficient inoculation volume.
- ii) Adsorb the specimen for 1 hour at 37°C.
- iii) Remove inoculum. Add fresh tissue culture medium containing 2% fetal calf serum and other required additives, as per specific medium and cell line requirements.
- iv) Incubate at 37°C and 5% CO<sub>2</sub> for 4–7 days.
- v) Test supernatant for presence of CCHFV viral RNA using real-time RT-PCR as described below, or perform immunofluorescence assay on cell scrapings.
- vi) Isolates of CCHFV from clinical specimens cause no microscopically recognisable cytopathic effects (CPE) in most of these cell lines.

## 1.2. Nucleic acid detection

Molecular-based diagnostic assays, such as RT-PCR, serve as the front-line tool in the diagnosis of CCHF, as well as other viral haemorrhagic fevers (Drosten *et al.*, 2003). The benefit of molecular diagnostic assays is their rapidity compared to virus culture, often allowing a presumptive diagnosis to be reported within a few hours after receiving a specimen (Burt *et al.*, 1998). The RT-PCR is a sensitive method for diagnosis, but because of genetic diversity of CCHFV, there might be some challenges with regard to design of primers or probes that allow detection of all circulating strains of the virus. A real-time RT-PCR that detects strains from different geographic locations has been recently described. The assay was shown to be highly sensitive; it can detect as little as 1,164 viral RNA copies per ml of plasma (Wölfel *et al.*, 2007). A low-density microarray developed on the basis of the most up-to-date genome information has been extensively validated in clinical specimens collected from confirmed cases of CCHF over 20 years by a WHO reference laboratory. It was shown to detect as few as 6.3 genome copies per reaction (Wölfel *et al.*, 2009).

A real-time RT-PCR which targets the S-segment of CCHFV genome and detects strains from different geographic locations was developed by Wölfel *et al.* (2007). The method employs an *in-vitro* transcribed RNA copy of the full S-segment as quantitative RNA standard. The assay is based on a pair of primers and three probes. The template used in the assay is viral RNA extracted from the specimen by using any standard viral RNA extraction method or commercially available kit. The sequences and binding sites for the primers and three probes are as follows:

### Primer RWCF

- i) Forward primer
- ii) Position 1068–1095
- iii) Sequence 5'-CAA-GGG-GTA-CCA-AGA-AAA-TGA-AGA-AGG-C-3'

### Primer RWCR

- i) Reverse primer
- ii) Position 1248–1223
- iii) Sequence 5'-GCC-ACA-GGG-ATT-GTT-CCA-AAG-CAG-AC-3'

**Probe SE01**

- i) Broad-range probe
- ii) Position 1172–1198
- iii) Sequence 5'-FAM-ATC-TAC-ATG-CAC-CCT-GCT-GTG-TTG-ACA-TAMRA-3'

**Probe SE03**

- i) Additional probe
- ii) Position 1172–1198
- iii) Sequence 5'-FAM-ATT-TAC-ATG-CAC-CCT-GCC-GTG-CTT-ACA-TAMRA-3'

**Probe SE0A**

- i) Additional probe
- ii) Position 1131–1106
- iii) Sequence 5'-FAM-AGC-TTC-TTC-CCC-CAC-TTC-ATT-GGA-GT-TAMRA-3'

The assay was validated using reagents from a specific commercial kit, but these can be substituted with any equivalent reagents from other commercial kits. The reaction is set up as follows. 25 µl reaction volume consisting of 5 µl viral RNA, 1× concentration of PCR reaction buffer and enzymes from any commercially available one-step RT-PCR kit, and 400 µmol dNTP, 800 ng non-acetylated bovine serum albumin. The cycling parameters are as follows: 30 minutes at 50°C, 15 minutes at 95°C, 46× 15 seconds at 94°C, 30 seconds at 59°C, and 30 seconds at 72°C. Fluorescence acquisition occurs at the 59°C step mode.

## 2. Serological tests

Virus neutralisation assays, generally considered to be highly specific, are rarely used for CCHFV diagnosis. Members of the *Nairovirus* genus generally induce a weaker neutralising antibody response than members of other genera of the family *Bunyaviridae*. Another drawback is the necessity to perform this assay in high biosafety containment because it uses live virus (Burt *et al.*, 1994; Rodriguez *et al.*, 1997).

Currently, there are only a few CCHFV commercial kits for IgM or IgG by ELISA or immunofluorescence (IFA). These are all designed for the human diagnostic market. However, it is possible to adapt these commercial ELISAs and IFAs for serological testing in animals. In addition, some in-house ELISAs have been published for the detection of CCHFV-specific antibodies in animals (Table 2).

ELISAs for CCHFV-specific IgM and IgG antibodies are specific and more sensitive than IFA. IgM antibodies in livestock (sheep, goat and cattle) can be detected by using an IgM-capture ELISA. IgG antibodies can be detected by an IgG-sandwich or indirect ELISA, and total antibodies can be detected by competition ELISA. The benefit of competitive ELISA is the capacity to investigate different animal species, because they are host species independent. The protocols of the published assays (Table 2) exemplify general procedures and protocols for different kind of assays for the detection of CCHFV-specific antibodies. The limiting factor for the replication of these protocols in other laboratories is the availability of antigens and (where relevant) specified monoclonal antibodies. Most of the tests described for livestock and wild animals have not undergone a formal validation process (Mertens *et al.*, 2013). One of the biggest challenges for such validation studies is the availability of an adequate number of positive well characterised control samples.

For information on the availability of reference reagents for use in veterinary diagnostic laboratories, contact the OIE Reference Laboratories or the OIE Collaborating Centre for Zoonoses in Europe.

**Table 2.** Serological assays for the detection of CCHFV-specific antibodies or the detection of viral antigen

Assay		Target species	Developer/producer of the assay
commercial assays	IgM ELISA	human	BDSL, Dreghorn, Scotland
	IgG ELISA		Vector-Best, Novosibirsk, Russia
	IgM IFA		Vector-Best, Novosibirsk, Russia
	IgG IFA		Euroimmun, Luebeck, Germany
	antigen capture ELISA	virus	Euroimmun, Luebeck, Germany
in-house assays	IgM ELISA	human	(Mourya <i>et al.</i> , 2012)
			(Garcia <i>et al.</i> , 2006)
			(Dowall <i>et al.</i> , 2012)
			(Emmerich <i>et al.</i> , 2010)
			(Tang <i>et al.</i> , 2003)
	IgG ELISA	human	(Burt <i>et al.</i> , 1994)
			(Dowall <i>et al.</i> , 2012)
			(Garcia <i>et al.</i> , 2006)
			(Burt <i>et al.</i> , 1994)
			(Emmerich <i>et al.</i> , 2010)
			(Samudzi <i>et al.</i> , 2012)
	IgM IFA	human	(Saijo <i>et al.</i> , 2002a)
			USAMRIID, Fort Detrick, USA
	IgG IFA	human	(Burt <i>et al.</i> , 1994)
			(Saijo <i>et al.</i> , 2002b)
IgG Western-Blot assay	human	(Burt <i>et al.</i> , 1994)	
		(Xia <i>et al.</i> , 2011)	
IgM ELISA	sheep,	(Xia <i>et al.</i> , 2011)	
		Burt <i>et al.</i> , 1993	
IgG ELISA	sheep	(Burt <i>et al.</i> , 1993)	
		(Qing <i>et al.</i> , 2003)	
	cattle	(Garcia <i>et al.</i> , 2006)	
		(Garcia <i>et al.</i> , 2006)	
animals	animals	CDC, Atlanta, USA	
		USAMRIID, Fort Detrick, USA	
competitive ELISA	species independent	(Burt <i>et al.</i> , 1993)	
antigen capture ELISA	virus	(Burt <i>et al.</i> , 1993)	
		(Saijo <i>et al.</i> , 2005)	

IFA: immunofluorescence assay. ELISA: enzyme-linked immunosorbent assay  
(Data and table modified from Mertens *et al.* 2013.)

### C. REQUIREMENTS FOR VACCINES

There is no vaccine available for animals.

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**NB:** There is currently (2021) there were no OIE Reference Laboratories for Crimean–Congo haemorrhagic fever (please consult the OIE Web site: <https://www.oie.int/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

**NB:** FIRST ADOPTED IN 2014.