Serological tests for Crimean-Congo haemorrhagic fever virus aid outbreak management

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By Dr. Jacqueline Gosink

Crimean-Congo haemorrhagic fever (CCHF) is a life-threatening tick-borne disease that is prioritised by the World Health Organization (WHO) for research and product development for early diagnostics. To address the need for serological assays for use in outbreak situations, EUROIMMUN has developed and evaluated ELISAs for the detection of IgM and IgG antibodies against the causative virus (CCHFV). The ELISAs reliably identified acute CCHF cases and are thus suitable for use in laboratories involved in on-site outbreak support.

Severe haemorrhagic disease

Crimean-Congo haemorrhagic fever (CCHF) is a highly contagious disease with a case fatality rate of up to 40 per cent, according to the WHO. The principal vector is ticks of the genus *Hyalomma*. Humans contract infections through tick bites or contact with blood or tissues of viraemic animals or humans, for example, through occupational work as farmers, slaughterers, veterinarians, or healthcare workers.

The estimated number of human CCHFV infections worldwide is 10,000 to 15,000 per year. The majority of infections remain subclinical, but some cases develop into severe or even fatal diseases. Initial symptoms are unspecific, for example, high fever, myalgia, headache, diarrhoea, nausea and vomiting. Patients may subsequently develop haemorrhagic manifestations in different parts of the body and organ failure. CCHF-associated haemorrhagic phenomena rank among the most severe of all haemorrhagic fevers.

Expanding geographical range

The endemic region of CCHFV encompasses countries in Africa, the Balkans, the Middle East and Asia that lie within the geographical habitat of *Hyalomma* ticks. Türkiye has the highest number of laboratory-confirmed CCHF cases worldwide. Climatic, ecologic and anthropogenic factors are increasing the spread of CCHF, for example in Europe. It is anticipated that ongoing climate change in the Mediterranean region will lead to an increase and northward expansion of *Hyalomma marginatum* habitat areas. In addition, changes in land permit the proliferation of vertebrate hosts and ticks may lead to increased CCHFV transmission.

WHO priority

Due to its high case fatality rate, its potential for nosocomial outbreaks, and the difficulties in prevention and treatment, CCHF is included in the WHO's R&D Blueprint for Action to Prevent Epidemics. There are currently no internationally approved CCHF vaccines or antivirals, and chemical tick control is only realistic in well-managed livestock facilities. Management of outbreaks, therefore, relies on rapid identification of cases and initiation of infection control measures to prevent secondary nosocomial and community-level transmission.

CCHF is difficult to distinguish clinically from other viral haemorrhagic fevers such as those caused by Ebola, Marburg, Lassa, Rift Valley, dengue and yellow fever viruses. Disease surveillance is further hindered by the absence of a universally accepted gold standard for CCHFV diagnostics and the lack of international reference reagents for calibrating and harmonising assays. Thus, validated CCHFV diagnostic assays for use in reference laboratories and point-of-care settings in CCHF-affected countries are urgently needed.

Laboratory diagnostics

A combination of molecular and serological testing has been proposed to maximise diagnostic sensitivity. Reverse transcription-polymerase chain reaction (RT-PCR) is used for diagnosing active CCHF at the early stage, as it enables sensitive detection of viral RNA. However, the molecular detection is impaired by the wide genetic diversity of CCHFV strains, and its application is limited to the viraemic phase. Results usually turn negative after the first few days of the disease, and only patients with a fatal outcome remain positive until death.

Serological assays provide a second pillar for identifying infections. The major target of the immune response against CCHFV is the viral nucleocapsid (N) protein. N-specific IgM becomes detectable around two to three days after onset of symptoms, followed by IgG one to two days later. IgM reaches peak levels in the second and third weeks of disease and usually clears after about four months, while IgG persists for several years. Severe and fatal infections, however, are often serologically undetectable due to a weak or absent antibody response. IgM assays are mainly employed to support diagnosis of acute infections, while IgG assays are relevant at later stages and for disease surveillance.

Reliable CCHFV ELISAs

The Anti-CCHFV ELISAs from EUROIMMUN utilise recombinant viral N protein as the antigenic substrate for detection of IgM and IgG antibodies against CCHFV. In a study published by Cosgun et al in the journal Vector-Borne and Zoonotic Diseases,, the diagnostic performance of the ELISAs was evaluated against established tests. These were the EUROIMMUN CCHFV Mosaic 2 IgM and IgG indirect immunofluorescence assays (IFA), which served as reference tests, and IgM and IgG ELISAs from another manufacturer.

The EUROIMMUN Anti-CCHFV IgM and IgG ELISAs both yielded a higher sensitivity (IgM 98.0 per cent, IgG 47.1 per cent) than the other manufacturer's ELISAs (IgM 95.9 per cent, IgG 35.3 per cent), as determined in 49 serum samples from patients with acute-phase CCHFV infection. The lower sensitivity of both IgG ELISAs compared to IFA may be due to differing antigen presentation between the two techniques and the study panel of acute-phase samples. The main value of IgG analysis is, however, in disease monitoring and epidemiological surveillance. In a further study published by Emmerich et al in the journal PLOS Neglected Tropical Diseases, the EUROIMMUN IgG ELISA demonstrated a sensitivity of 98.8 per cent in patients with subsided CCHF.

The specificity of the EUROIMMUN IgM ELISA was slightly higher (86.4 per cent) than that of the other ELISA (84.7 per cent), as evaluated in 89 sera from control patients, healthy blood donors and patients with hantavirus or sandfly fever infections. For IgG, both assays demonstrated a specificity of 100 per cent. The ELISAs from the two manufacturers thus show a substantial qualitative agreement and a very strong positive correlation of quantitative results for both IgM and IgG.

Outlook

Efficient CCHF surveillance requires reliable, simple-to-use, validated, and easily accessible diagnostic assays, enabling local laboratories to perform fast serodiagnosis at moderate biosafety levels. Analysis at local facilities bypasses the need to send patient samples to national or international reference laboratories, which increases the time and costs per analysis and involves the shipment of highly contagious material.

> **A combination of molecular and serological testing can maximise diagnostic sensitivity.**

ELISAs are less complex than other serological methods, making them highly suitable for use in outbreak situations. The EUROIMMUN Anti-CCHFV ELISAs have proven to be standardised and easy-to-use tools that reliably detect acute CCHF cases. They can be fully automated on established instruments, providing increased standardisation and efficiency. In addition to their diagnostic application, serological tests are also useful for epidemiological studies in endemic areas, monitoring virus emergence in neighbouring regions, and evaluating vaccine immunogenicity and durability. ●

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