MOLECULAR BIOLOGY TECHNIQUES USED IN ACTIVE SURVEILLANCE OF CRIMEAN–CONGO HAEMORRHAGIC FEVER IN RUMINANTS: A CRITICAL REVIEW

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic disease with high risk of emergence in areas with uncontrolled high population of ixodid or argasid ticks. Disease is caused by a Nairobi virus (family Bunyaviridae), one of the three known humans’ pathogenic viruses of the genus, along with Dugbe virus and Nairobi sheep disease virus. Despite the important role played by livestock in the transmission and amplification of the CCHF virus, active surveillance of the disease is not part of the current actions of disease control in several countries. This situation may be due to subclinical evolution in animals and no economic effects on the livestock products. Nevertheless, CCHF virus is a major pathogen for humans, with haemorrhagic manifestations and mortality rate ranging from 5% to 80%. Consequently, implementation of public health measures in areas with high risk is decisive. This review summarized virus identification and immunological methods designed for CCHF and that may be used in active surveillance of disease in ruminants. Diagnostic tests for virus identification in animals can be used for surveillance (virus isolation in cell culture) or for determining the virus-free status (real-time reverse transcription polymerase chain reaction) In conclusion the front-line tool in diagnosis of CCHF seen to be reverse transcriptase polymerase chain reaction, and until the validation of serological methods seems to be the better for active surveillance of CCHF in ruminants.

Key words: CCHF, tick-borne zoonotic disease, epidemiology, serological surveillance, RT-PCR.

INTRODUCTION

Crimean-Congo haemorrhagic fever (CCHF) is a tick-borne zoonotic disease, endemic in Africa, Asia and Eastern Europe, including the Balkan Peninsula (Papa et al., 2002, 2004; Ahmeti, 2006), overlapping the distribution area of Hyalomma spp. (Appannavar and Mishra, 2011).

CCHF virus (CCHFV) is a Nairobi virus (family Bunyaviridae), being one of the three viruses pathogenic for humans, belonging to this genus, along with Dugbe virus and Nairobi disease virus (Swanepeol and Pavjeska, 2011). CCHFV has a spherical capsid (≏100 nm) harbouring a genome single-stranded RNA, segmented in three linear fragments (large, medium and small) negatives, which encode: viral nucleocapsid (N), the precursor of the glycoprotein (GPC), and of the polymerase (L) (Schmaljohn and Hooper, 2001; Briese et al., 2013).

Life cycle of CCHFV can be hosted either by the arthropod-arthropod (transovarial and transstadiol) chain, either by the arthropod-vertebrate-arthropods chain. The ticks can suck blood on several wild and domestic animals, and, the last ones, in their turn, can infect other ticks. Also, the human can be exposed to the CCHF virus by direct contact with the blood or the tissues of the infected animal. (Kamboj and Pathak, 2013; OIE, 2014).

CCHFV has high risk of emergence in areas where the control policy of ixodid or argasid ticks is missing. (Bajpai and Nadkar, 2011).
Despite the important role played by livestock in the transmission and amplification of the CCHFV, most countries do not have sanitary veterinary programs for active surveillance of CCHF in animals. This situation may be due to the subclinical evolution in animals and also, to the weak impact on the economic performances in the livestock industry (OIE, 2014). Despite the effects on the animals, CCHFV is a major pathogen for humans. Clinical signs in humans consist in fever, chills, severe headache, dizziness, photophobia, myalgia and arthralgia, ecchymosis, hematemeses, melena, epistaxis, haematuria, and haemoptysis (Kamboj and Pathak, 2013). Mortality rate is ranging from 5% to 80% (OIE, 2014).

In Balkans, CCHF is a zoonotic disease emerging, with high risk of exposure to this virus for animal workers, for animal handlers, for butchers, for the veterinarians, hunters, and foresters (Papa et al., 2002, 2004; Ahmeti and Raka, 2006; OIE, 2014).

**PROBLEM FORMULATION**

According of the above data, the implementation of public health measures in European states should be considered. This paper summarizes the available tools for the virus identification and immunological methods designed for CCHF and that may be used in active surveillance of disease in ruminants.

In order to propose the most appropriate molecular technique for the active surveillance of CCHF in ruminants, we compared the results reported by 19 teams of researchers in 23 scientific reports and reviews which used molecular biology techniques in detection of CCHFV, using different types of samples (Rodriguez et al., 1997; Burt et al., 1998; Drosten et al., 2002, 2003; Yashina et al., 2003; Nabeth et al., 2004; Yapar et al., 2005; Tonbak et al., 2006; Papa et al., 2004, 2007; Duh et al., 2007; Midilli et al., 2007; Wölfel et al., 2007, 2009; Deyde et al., 2009; Grard et al., 2011; Weidmann et al., 2011; Atkinson et al., 2012; Lindeborg et al., 2012; Al-Zadjali et al., 2013; Fajs et al., 2014; Kamboj et al., 2014; OIE, 2014).

**PROBLEM SOLUTION**

To set up preventive programs and therapeutic protocols for CCHFV infections, the first request to satisfy is the early diagnosis. (Al-Zadjali et al., 2013). An ideal preventive program should prevent human cases of disease, by keeping the infection only in animals or in vectors, at his lowest level and focusing the surveillance on the CCHFV screening in domestic or wild animals. Until today, the disease surveillance is carried out by various virological methods, like CCHFV isolation (on cell cultures) or (reverse transcription PCR) (OIE, 2014).

The choice of one or other of method has different reasons: by serology can be counted the immune outcome to infection, meaning retrospective diagnosis, while the virus detection and the viral load by quantitative real-time PCR can be used in prediction of the clinical progress. (Duh et al., 2007; Papa et al., 2007; Wölfel et al., 2007).

Several serological methods (e.g. ELISA and immunofluorescence assay) are available to characterise the status of infection of a subject or of the population, or the prevalence of infection (a reliable indicator of virus circulation on the field) (OIE, 2014). In human infection, the tests used in quantification of IgM could not be used as a predictor of disease outcome because the levels of IgM were not correlated with the outcome of viral load, while levels of IgG were inversely correlated with viral loads (IgG might neutralize the virus) (Duh et al., 2007). All marketed kits, ELISA or IFA, are designed only for human samples, while for ruminants have been designed only in-house ELISA protocols. These tests have not undergone a formal validation process (OIE, 2014).

Real-time PCR for the small (S) CCHFV segment (amplifying a 127-bp product) (Wölfel et al., 2007) was used in monitoring the influx of migratory birds carrying CCHFV infected ticks; this study supports that the real-time PCR can be a tool for predicting the risk of emergence of new CCHFV foci (Lindeborg et al., 2012).

In India, Appannanavar and Mishra (2011) emphasizes the need of active surveillance not only for existing pathogens in any geographic
location (e.g. Ganjam virus of Nairobi sheep disease) but also for those that pose future threat. They recommend real-time PCR in CCHFV surveillance instead of Serological tests (Appannanavar and Mishra, 2011).

Drosten et al. (2002) evaluated several viral hemorrhagic fevers by real-Time reverse Transcription-PCR and identified some problems that can impair the sensitivity and specificity of molecular biology diagnostic. For instance, in design of a multiplex PCR is difficult to achieve optimal reaction conditions for each individual pair of primers used for each virus. Therefore, Drosten et al. (2002) recommend additional tests (e.g. virus isolation, ELISA, singleplex PCRs, IgM/IgG detection by ELISA or IFA) in confirmation of a human case of CCHF (Drosten et al., 2002). Reliable, specific, sensitive and simple RT-PCR assays were described by Rodriguez et al. (1997), Burt et al. (1998), Drosten et al. (2002), Yashina et al. (2003), Yaper et al. (2005), Tonbak et al. (2006), Midilli et al. (2007), Deyde et al. (2009), and Grard et al. (2011), some of them assessed and certified in complex molecular investigations and genetic analysis. Low-Density Microarray for the Rapid Detection and Identification of the CCHFV was already described, and the results seem to exceed those of real-time PCR protocols (Wölfel et al., 2009), but CCHF virus strains cannot be completely characterised by this method. Unfortunately, this technology is running only in few diagnostic laboratories and its implementation in surveillance or diagnostic is still expected.

CONCLUSIONS

According to data above presented, the frontline tool in CCHF diagnosis is the reverse transcription polymerase chain reaction and, as well as in humans, can be used successfully in ruminants. Also, absence of validated serological methods for active surveillance of CCHF in ruminants, support the introduction of reverse transcription polymerase chain reaction in surveillance programs.

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