

ISOLATION OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS FROM TICKS ON IMPORTED SUDANESE SHEEP IN SAUDI ARABIA

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Crimean-Congo hemorrhagic fever (C-CHF) is a viral tick-borne zoonosis which occurs in Europe, Asia and Africa.¹ The virus is a member of the genus *Nairovirus*, family Bunyviridae, and circulates between ixodid ticks and mammals, including man and domesticated animals. Human infection is characterized by hemorrhagic signs and fever, the same symptoms which appear in some other viral diseases such as Lassa fever, Marburg and Ebola,² and which have the same mode of transmission (case to case transmission) as C-CHF.

An outbreak of C-CHF occurred in the Western region of Saudi Arabia in 1990. It was suspected at the time that the disease had been introduced to the region through the Jeddah seaport via tick-infested imported animals.³ Screening tests of imported livestock for detection of immunofluorescence IgG to C-CHF proved positive for both Sudanese sheep and goats.

The findings prompted a sero-survey study of imported livestock arriving at the Jeddah seaport, using the reversed passive hemagglutination inhibition (RPHI) test, which is reliable and sensitive in the detection of viremic C-CHF cases. The study showed that the small Sudanese ruminants had the highest C-CHF antibody prevalence.⁴ The antibodies were detected in 4.1% of examined sheep and 3.2% of goats. This report represents the results of virus isolation trials from ticks on Sudanese sheep arriving at Jeddah seaport from March to October 1995.

Materials and Methods

Shipments of sheep from Sudan arriving at the Jeddah seaport were examined for tick infestation. When the shipment was found positive, a number of sheep were randomly selected for tick collection by hand. The collected adult ticks were pooled alive in plastic containers with a cement layer and a piece of wet filter paper at the bottom. The containers were then tightly sealed with gauze and transferred to a veterinary diagnostic laboratory for identification. Virus isolation was carried out with the help

of the Virology Division, Research Center, King Abdulaziz University, Jeddah.

Antigens of C-CHF virus and antisera were obtained from the Centers for Disease Control (CDC), Atlanta, US. The antigens were prepared by sucrose-acetone extraction of infected mouse brain. Antisera were in the form of mouse immune ascitic fluids (MIAF). Stock cultures of Vero cells were grown in Eagle mineral essential amino acid medium, with Hanks salt solution (EMEM) supplemented with 10% fetal bovine serum and antibiotics.

Pools of ticks were ground in a mortar to approximately 10% in cell culture medium containing antibiotics. The suspensions were centrifuged at 10,000 rpm for 10 minutes, and the supernatant was inoculated into 2-3 days' old mice intracerebrally (5 mice/pool), and into the cell culture of Vero cells. The mice were observed for two weeks. If abnormal or dead mice were found, their brains were removed for inoculation into Vero cells and for virus identification.

The isolation of the C-CHF virus was confirmed by: 1) indirect immunofluorescence on impression smears from the brain of affected mice, as described by Shepherd et al.⁵, and 2) the homogenization of the brain of affected mice to 10% (wt/vol) in EMEM and clarified by centrifugation at 10,000 rpm for 30 minutes. Extract supernatants were tested for the virus antigen by immunodiffusion, as described by Clarke,⁶ and ELISA, as described by Shepherd et al.⁵

Results

Four shipments of Sudanese sheep were found to be infested with ticks. C-CHF virus was isolated from three out of eight pools of ticks (Table 1). The first pool had 25 specimens of *Hyalomma* species. The other two pools contained more than one genus of ticks. The second pool included 28 specimens, comprising 10 *Boophilus* sp., 8 *Rhipicephalus* sp., 8 *Hyalomma* sp. and 2 *Amblyomma* sp. The third pool comprised 20 specimens, comprising 8 *Hyalomma* sp., 7 *Boophilus* sp. and 5 *Rhipicephalus* sp. The ticks of the first pool were retrieved from ears and the inguinal region, whereas those of the second and third pools were found predominantly on the head and ears and a few between the thighs and around the anus. The ticks were slightly to fully engorged.

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TABLE 1. C-CHF virus isolation trials from ticks collected from four shipments of Sudanese sheep at Jeddah seaport, Saudi Arabia.

Total number of sheep/shipment	No. of sheep deticked	No. of pools collected	No. of pools positive	No. of ticks collected
5120	7	1	1	25* (25)**
2128	6	2	2	48 (20, 28)
3435	4	2	0	36 (20, 16)
7500	10	3	0	75 (25, 25, 25)

*Total number of individuals collected; ** total number of individuals/pool.

In the three positive pools, 5 of the 15 inoculated newborn mice died within 5-8 days. The virus was not successfully isolated from ticks in tissue culture, but it could be isolated on Vero cells from infected mice.

Discussion

The seropositivity of C-CHF virus among small Sudanese ruminants in a previous study⁴ and the isolation of the virus from ticks on Sudanese sheep in this study indicate that the C-CHF virus occurs in Sudan. Ecologically and geographically, the occurrence of C-CHF virus in Sudan is not surprising. Sudan lies predominantly in the Ethiopian zoogeographic zone where the virus thrives, and borders countries where the virus is prevalent.¹ In addition, the tick fauna of Sudan includes many species which are capable of transmitting the virus.⁷

The detection of C-CHF infection in ticks and animals imported from Sudan also indicates that the virus could be transmitted to Saudi Arabia. Small Sudanese ruminants mingle with the local breeds in markets and farms, providing the opportunity for the spread of the infection. As well, the infestation of Saudi animals with ticks helped in supporting the establishment of the virus.³

It seems that the C-CHF virus may be more widely distributed in the Middle East and North Africa than presently recorded. These regions provide a favorable habitat for the virus, with a variety of potential vectors and diversity of wild and domestic vertebrate hosts. The presence of the disease is only noticed when human cases of infection appeared, as it is asymptomatic in animals. Undetected illnesses may perhaps occur in remote areas of the Middle East and North Africa, where there is a paucity of healthcare facilities and lack of awareness of C-CHF risks. Recently, the disease was recorded in Saudi Arabia³ and in Oman,⁸ and previously, in Iraq⁹ and in the United Arab Emirates.¹⁰ In Kuwait,¹¹ antibodies to C-CHF virus were recorded in human populations, indicating previous exposure to the virus, however, no investigations on ticks or sheep, which are better indicators of the intensity and circulation of the virus, were carried out. In Egypt, C-CHF antibody was detected in livestock and in one man,¹² but the virus has not been isolated, and neither was the human case recorded. Further investigations are needed to give a clear and complete picture of the ecology and epidemiology of the virus in the Middle East and North Africa.

Despite the presence of C-CHF in Sudan, cases of human infection by the virus have not been recorded.

Similar observations have been reported in other countries such as Greece¹³ and Niger,¹⁴ where the virus and/or antibodies were detected. In South Africa, despite indication of high levels of virus transmission in livestock and frequent human exposure to ticks and blood from domestic animals, cases of human infection have been infrequent.¹⁵ This suggests that man is an accidental host, only infected by chance and under certain circumstances, while the main circulation of the virus in nature is among ticks and animals.

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