A SEROLOGICAL SURVEY IN SUSPECTED HUMAN PATIENTS OF CRIMEAN-CONGO HEMORRHAGIC FEVER IN IRAN BY DETERMINATION OF IGM-SPECIFIC ELISA METHOD DURING 2000 - 2004


Crimean-Congo hemorrhagic fever is a zoonotic arboviral disease, which is transmitted to humans via tick bite or via human or domestic animal blood transmission, in particular sheep, goats, and cows.

Keywords: Crimean-Congo hemorrhagic fever • Iran • serology

Introduction

Crimean-Congo hemorrhagic fever (CCHF) virus (genus Nairovirus, family Bunyaviridae) is widely distributed in wild and domestic mammals, birds, and ticks throughout many regions of Africa, Europe, and Asia. It was first observed in the Crimea by Russian scientists in 1944.

The virus was first isolated in Africa in 1956 from the blood of a febrile patient in Zaire. It has been said that transmission may also occur through direct or aerosol contact with the blood of patients in advanced stages of the disease. The primary group of vectors responsible for human disease appears to be several species of the genus Hyalomma.

Clinical CCHF was first recognized in Iran in 1999 with occurrence of the disease in several unrelated cases in different provinces, mainly Chaharmahal and Bakhtiari. Infection with CCHF virus was confirmed with the ELISA technique in a few patients when their sera were sent to the South African Arboviral Laboratory Research and Reference Center. After that the Laboratory of Research and Diagnosis on Arboviruses and Viral Hemorrhagic Fevers (National Center), which is unique in Iran and in the region, was established in October 2000 at the Pasteur Institute of Iran on the basis of public health necessities. Since then, suspected human and domestic animal serum samples from all parts of Iran, are sent to this laboratory and analyzed by ELISA method and RT-PCR technique. The present study is a brief report of our research on CCHF in serological field.

The aim of this study was to characterize the situation of CCHF virus infection in humans involved in animal-contact occupations in Iran between 2000 to 2004. Supporting objectives were to identify CCHF antibody-positive IgM in humans.

Materials and Methods

One-thousand three-hundred ninety-four serum
samples from 683 suspected human patients were collected between June 7th, 2000 and October 15th, 2004 from suspected patients who had fever, thrombocytopenia, and hemorrhagic symptoms or who had a recent travel history to endemic areas.

The sera samples collected from individuals were examined by enzyme-linked immunosorbent assay (ELISA) for (IgM) antibody to CCHF virus in suspected patients at the Pasteur Institute of Iran.

IgM detection was done as follows: the ELISA plates were coated with goat IgG fraction to human IgM diluted in phosphate-buffered saline (PBS) 1x and incubated overnight at 4°C. After that the sera, diluted in PBS containing 0.05% Tween (PBST) and 3% skim milk (PBSTM), were added and the plates were incubated for 1 hour at 37°C. Then, antigen diluted in PBSTM was added and the plates were incubated for 3 hours at 37°C. After that immune ascites (IA) diluted in PBSTM was added and the plates were incubated for 1 hour at 37°C. After all incubations, the plates washed 3 times with PBST. Finally, hydrogen peroxide (H2O2) and 3, 3', 5, 5' tetramethyl benzidine (TMB) was added and the plates were incubated for 15 minutes at room temperature. The enzymatic reaction was stopped by the addition of 4N H2SO4 ELISA reader read the plates at 450 nm. The data were analysed by SPSS version 10.0.

**Results**

In this study, the seroepidemiology of CCHF in different provinces of Iran was investigated during 2000 – 2004.

Seven-hundred and eighty-three suspected patients from various Iranian provinces were examined and 248 patients were IgM positive for CCHF. Among those who were IgM positive, 24 persons died. The results are shown in Table 1.

**Discussion**

CCHF infection is reported from many countries, in particular in the Persian Gulf region 5, 7, 10, 13, 16, 18 but also from countries on various continents. 14

The characteristics of CCHF virus infection in domestic animals are often unapparent, while human infections often result in severe hemorrhagic fevers. 8, 9

The incubation period for CCHF virus infection in humans is commonly 1 week, but varies depending on the type of exposure (tick bite, contact with blood, or skin of infected animals). Viruses are only detectable during the first week of illness so analysis of the humoral response is the best mode of detection for the disease.

The patients were from 14 of the 28 provinces of Iran. However, the majority of the confirmed cases were from Sistan and Balochestan, which is located in neighborhoods of Pakistan and Afghanistan.

As it has been shown in Table 1, we have also found CCHF-positive cases in the following provinces: Isfahan, Fars, Golestan, Khozestan, Tehran, Khorasan, Boshehr, Lorestan, Yazd, Hormozgan, Kordestan, Markazi, and Chaharmahal and Bakhtiari.

It is noteworthy to mention that human sera were tested for 4 other types of viral fevers i.e. yellow fever, Rift Valley fever (RVF), Dengue 2,
and Chikungunya. It should be noted that no specific antibodies against any of these viral fevers were detected in analyzed sera.

Thus, our results indicate that CCHF virus is the principal agent of the hemorrhagic fevers in Iran. Among different modes of transmission, the made of CCHF virus transmission in Iran is not completely clear, but it seems handling the infected organs of slaughtered livestock may be the main way of contamination in Iran.

It can be concluded that 36.31% of suspected patients were IgM positive for CCHF and, 27 (10.89%) of the 248 IgM-positive patients subsequently died.

Native antigen was previously used for ELISA tests, which was prepared from brain of newborn mouse in Biosafety Level 4 Laboratories and sent to us as a result of international Pasteur Interdepartmental collaborations. Recently, a recombinant antigen has been produced by scientific collaboration between Laboratory of Arboviruses and Viral Hemorrhagic Fevers of Pasteur Institute of Iran and “Unit Postulante de Genetique Imoleculaire des Bunyavirides” in Pasteur Institute of Paris. It is worth knowing that there is no need for a laboratory with level 4 of biosafety to be produced and, therefore, level 2 of biosafety can be used instead. The recombinant antigen has same functional activity for IgM and IgG detection by ELISA method for human and animal sera.

In the scientific collaboration between Arboviruses laboratory and Swedish Institute for Infectious Disease Control, the CCHF genome was analyzed for detection of CCHF virus sequences. The phylogenic study done in this collaboration showed that the new Iranian CCHF virus is similar to the strain in Pakistan and Madagascar, and it is different from the previous virus in Iran. Therefore at least 2 strains of CCHF exist in Iran.

References


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