CHAPTER 8

CRIMEAN-CONGO HEMORRHAGIC FEVER INFECTION IN IRAN

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8.1. INTRODUCTION

8.1.1. History of crimean-congo hemorrhagic fever infection in Iran

Crimean-Congo hemorrhagic fever (CCHF) disease existed from many years in the northwest of Iran, in Ardebil, East and West Azerbaijan provinces with the local name "Kara-Mikh typhoid Fever". Kara-Mikh is a Turkish word which means black nail and it refers to the black spots appearing on the skin of the patients.

In 1974, Dr. Asefi studied clinically 60 patients with CCHF syndromes in Ardebil, Sarab, and Khalkhal counties of the Ardebil province [1]. In the years 1974–1975, Dr. Ardoin from the Pasteur Institute of Paris, with the collaboration of Dr. Younis Karimi from the Pasteur Institute of Iran, has studied clinically the disease in East Azerbaijan province [2]. In 1975, Dr. Saidi et al. have demonstrated the presence of antibodies against CCHF in the blood of human, domestic animals, and small mammals suspected for the disease in different regions of Iran mainly the region bordering the Caspian Sea and East Azerbaijan [23]. In 1978, professor Sureau from the Pasteur Institute of Paris with collaboration of the Pasteur Institute of Iran succeeded to isolate the virus of the disease from infected ticks in Khorassan province in northeast of Iran [25]. After that time, because there were no facilities, no research has been done on this disease in Iran and patients with hemorrhagic diseases have been misdiagnosed as they were previously when CCHF was considered to be "Kara-Mikh typhoid Fever" and unfortunately some of them died without diagnosis and efficient treatment. In summer 1999, suspected cases for this disease have been reported in Iran and with the sending of the sera of suspected patients from August 1999 to June 2000 to the National Center of Virology in South Africa, 7 Out of 34 persons have been diagnosed positive (Table 8-1).

O. Ergonul and C. A. Whitehouse (eds.), Crimean-Congo Hemorrhagic Fever, 89–98.

Table 8-1. The characteristics of CCHF-diagnosed patients in 1999

Cases	Sex	Age	Profession	Province	Positive test result
Case 1	Female	24	Housewife	Khuzestan	ELISA IgG+
Case 2	Male	32	Physician	Chaharmahal Bakhtiari	ELISA IgM+, IgG+, IF+
[a]Case 3	Female	26	Physician	Chaharmahal Bakhtiari	ELISA IgM+, IgG+, IF+, Virus culture+
[a]Case 4	Male	25	Livestock dealer	West Azerbaijan	ELISA IgG+
Case 5	Female	29	Housewife	Khuzestan	RT-PCR+
[a]Case 6	Male	55	Farmer	Khuzestan	Virus culture+
Case 7	Male	37	Worker	Khuzestan	ELISA IgM+, IgG+

[a] These patients have died

8.2. RESPONSE TO CCHF OUTBREAK

Following these finding, in 2000, CCHF was recognized as a major public health problem by sanitary authorities in Iran and thus the Laboratory of Arboviruses and Viral Haemorrhagic Fevers (National Center) has been founded in the Pasteur Institute of Iran which is a unique center for research and diagnosis of Arboviruses in the region and this laboratory is well equipped for the serological and molecular diagnosis of this disease and other arboviral and hemorrhagic fever diseases [3, 4, 8, 9]. The Laboratory of Arboviruses and Viral Haemorrhagic Fevers of the Pasteur Institute of Iran has provided the entire health system of the country with the collaboration of the Ministry of Health of Iran a protocol according to which human serum samples suspected of CCHF disease are sent from different provinces of Iran respecting the cold chain [10, 13, 24] (Fig. 8-1).

From each patient, three serum samples must be prepared.

1. Following the clinical diagnosis of the patient: for reverse transcription polymerase chain reaction (RT-PCR) (detection of a fragment in the S segment of the virus genome) and specific enzyme-linked immunosorbent assay (ELISA) for detection of anti-CCHF IgM and anti-CCHF IgG (if it exists)
2. 5–10 days after the onset of the first clinical signs for specific IgM and IgG ELISA
3. 10–15 days after the onset of the first clinical signs for specific IgM and IgG ELISA

In the survey carried out from 7 June 2000 until 6 September 2005 by our lab, 1,528 samples from 763 suspected patients from different provinces of Iran have been tested serologically (specific ELISA) and molecularly (RT-PCR) [5, 7]. The results have been regularly reported to the Promed organization [14–22] (Table 8-2).

Around 763 CCHF-suspected patients (which means they had a sudden fever, at least one sort of hemorrhage and thrombocytopenia: less than 100,000 platelets/mL), 295 (262 + 33) were confirmed cases which means they were either IgM-positive or RT-PCR-positive for CCHF.

Confirmed Case

Confirmed fatalities

Fig. 8-1. The CCHF-infected provinces of Iran.

Table 8-2. The CCHF cases by the year 2005

Issues	Number
Samples	1,528
Suspected patients	763
IgM+ patients	262
IgG+ patients	245
RT+ and IgM+ patients	47[a]
Only RT+ patients	33
Total confirmed cases	295 (262 + 33)
Total confirmed death	53

[a] These 47 patients are comprised in the 262 IgM+ patients

After analyzing the profession of the infected patients, it is obvious that the most exposed profession to the infection is profession in which the person is in contact with livestock (farmer, worker, and truck driver) or has to slaughter them (butcher and slaughterer). Many cases are housewives who lived in the countryside and had contact with livestock (Table 8-3).

Our results show that the most infected province of Iran is Sistan and Baluchistan in the southeast of the country. This province has long border with Afghanistan and Pakistan, infected countries from which illegal entrance of humans and livestock occur into Iran (Table 8-4).

Table 8-3. The age, sex, and profession of the confirmed patients

		Number of confirmed cases
Sex	Male	233
	Female	62
Age group (year)	0–20	60
	21–40	157
	41–60	58
	61–80	20
Profession	Farmer	61
	Worker	54
	Housewife	52
	Butcher	38
	student	27
	Slaughterer	16
	Truck driver	9
	Employee	6
	Jobless	6
	Teacher	4
	Soldier	3
	Animal seller	3
	Health worker	2
	Others	14

Table 8-4. The CCHF-infected provinces of Iran

Name of the province	Confirmed case	Confirmed fatal case
Sistan and Baluchistan	184	17
Isfahan	34	9
Fars	19	5
Tehran	9	3
Khuzistan	8	1
Golestan	7	0
Khorassan	7	2
Lorestan	4	2
Boushehr	4	1
Yazd	3	1
Hormozgan	3	2
Markazi	2	2
Qom	2	2
Semnan	2	2
Zanjan	1	1
East Azerbaijan	1	1
Hamedan	1	1
Gilan	1	1
Kordestan	1	0
West Azerbaijan	1	0
ChaharMahal and Bakhtiari	1	0

8.3. NOSOCOMIAL CASES

In Iran, there are nosocomial cases. As mentioned in previous paragraph, there was a lady physician who caught the disease from her husband, also a physician who contracted the disease after contact with a hospitalized patient, and died from CCHF in 1999 in Chahar Mahal Bakhtiari province.

In 2001, another physician also caught the disease from his patients (a father and son), both butchers hospitalized as CCHF-suspected in the city of Isfahan and later confirmed as CCHF-positive. The physician fortunately recovered from the disease.

8.4. COMMENTS ON THE CASE FATALITY RATE

Among the 295 confirmed cases, 53 died because of CCHF. The case fatality rate (CFR) is 17.9% which is much lower than the reported CFRs from different countries. This could be related to the rapid diagnosis by serological and molecular techniques performed in the Laboratory of Arboviruses and Viral Haemorrhagic Fevers of the Pasteur Institute of Iran (National Center) and also in part to the awareness of Iranian clinicians, who begin ribavirin as soon as after the suspicion of CCHF. There is a good interdepartment collaboration between our lab and the Center for Disease Control (CDC) of Iran for rapid case finding, diagnosis, and report of the results of the patients.

8.5. ECOLOGIC FEATURES OF THE ENDEMIC REGION IN IRAN

Most positive cases are from Sistan–Baluchestan province (southeast of Iran) which has long borders with Afghanistan and Pakistan, two countries in which CCHF is endemic. Sistan–Baluchestan is an arid region with a windy and dry climate. Due to the climate of this province, agriculture and livestock breeding is very difficult. The transport of livestock is seen in the borders of this province from neighboring countries such as Pakistan and Afghanistan. During the transport, livestock infected with this virus and also with ticks containing this virus easily enter into this province. Livestock market in Zahedan (capital of Sistan–Baluchistan province) is full of these livestock (cattle, sheep, goat, and camel). A significant number of humans also live in close proximity of the livestock so they are at a great risk to catch the disease.

8.6. THE PROPOSED SOURCE OF INFECTION

The proposed source of infection is the entrance of infected livestock from the eastern borders of the country and their dispatch throughout the country where numerous humans can catch the disease by handling them directly or their carcass and by manipulation of their blood and discharges. Direct infection of humans by tick bite is rare in Iran and has been observed only in few cases.

8.7. PHYLOGENETIC STUDY OF IRANIAN CCHFV

In a joint study by the Laboratory of Arboviruses and Viral Haemorrhagic Fevers (National Center), Pasteur Institute of Iran and the Center for Microbiological Preparedness, Swedish Institute for Infectious Disease Control, CCHFV genome fragments for nine Iranian patients infected during 2002 were examined genetically nucleotide sequencing of the S and M segments, encoding the nucleocapsid protein (NP) and the glycoproteins, respectively. The study revealed that the different isolates were closely related to each other with nucleotide identities exceeding 98% for both S and M segments.

Phylogenetic analysis using S-segment sequences demonstrated that the Iranian isolates formed a distinct lineage together with the Pakistanian strain Matin and the Madagascar strain ArMg851. The Iranian strains analyzed in this study are clearly separated from a previously published Iranian CCHFV strain ArTeh193-3, indicating that at least two genetic lineages of CCHFV could be cocirculating in Iran (Fig. 8-2). Using M-segment sequences, it was confirmed that the Iranian strains examined in this study formed a separate cluster (Fig. 8-3). Interestingly, on the M tree the Pakistanian strain Matin was located outside the cluster formed by the Iranian isolates. These observations might indicate that some kind of genetic exchange could occur between different strains of CCHFV. Genetic reassortment has previously been demonstrated both in vivo and in vitro within the Bunyaviridae family among different members of the genera Bunyavirus and Hantavirus. It is not known at present whether members within the genus *Nairovirus* have the same capacity. These findings could suggest a possibility for genetic reassortment within CCHFV, but further studies are needed to substantiate this hypothesis. The Iranian sequences reported in this study have been the deposited into GenBank nucleotide sequence database with the following accession numbers: for the CCHFV S-segment sequences; AY366373–AY366379, and for CCHFV M-segment sequences; AY366380–AY366387 [6].

8.8. TREATMENT

According to the protocol of the CDC of Iran, *suspected patient* is a patient who has sudden onset of fever, myalgia, bleeding, one of the epidemiological signs such as tick bite or hand crushing of ticks or contact with fresh blood or other tissues of infected domestic animals, direct contact with the blood, and excretions of a confirmed or suspected patient of CCHF. A *probable case* is a patient who is a suspected case with a thrombocytopenia (less than 150,000 platelets/mm^3) with leucopenia (less than 3,000 lymphocytes/mm^3) or leucocytosis (more than 9,000 lymphocytes/mm^3). A *definitive case* is a patient who is a probable case with positive serological test or RT-PCR-positive [26].

Immediately after the diagnosis of the probable case, the patient is treated 10 days with ribavirin (ribavirin is used in pill form in Iran and other forms of

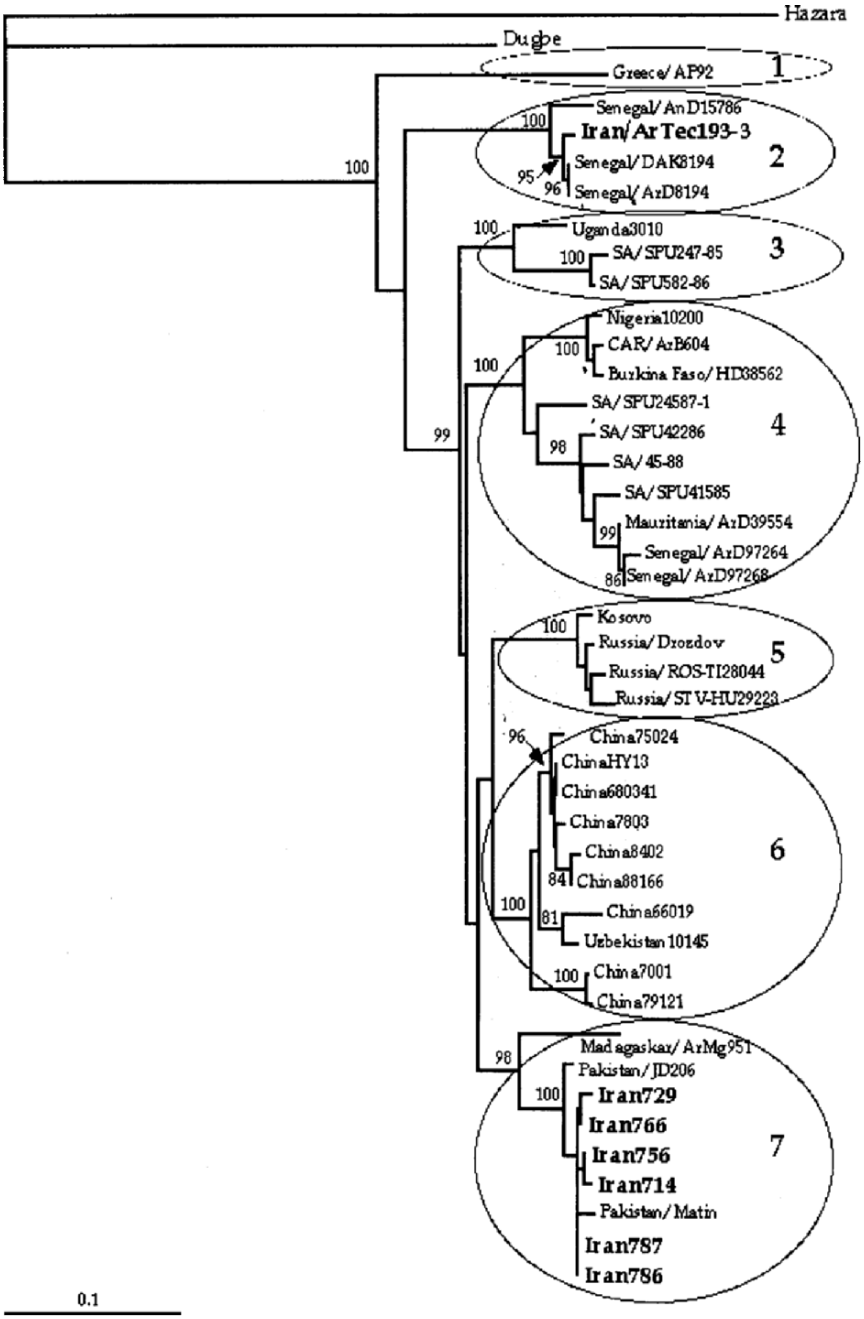

Fig. 8-2. Phylogenetic tree (neighbor-joining) calculated for Iranian CCHFV S-segment sequences.

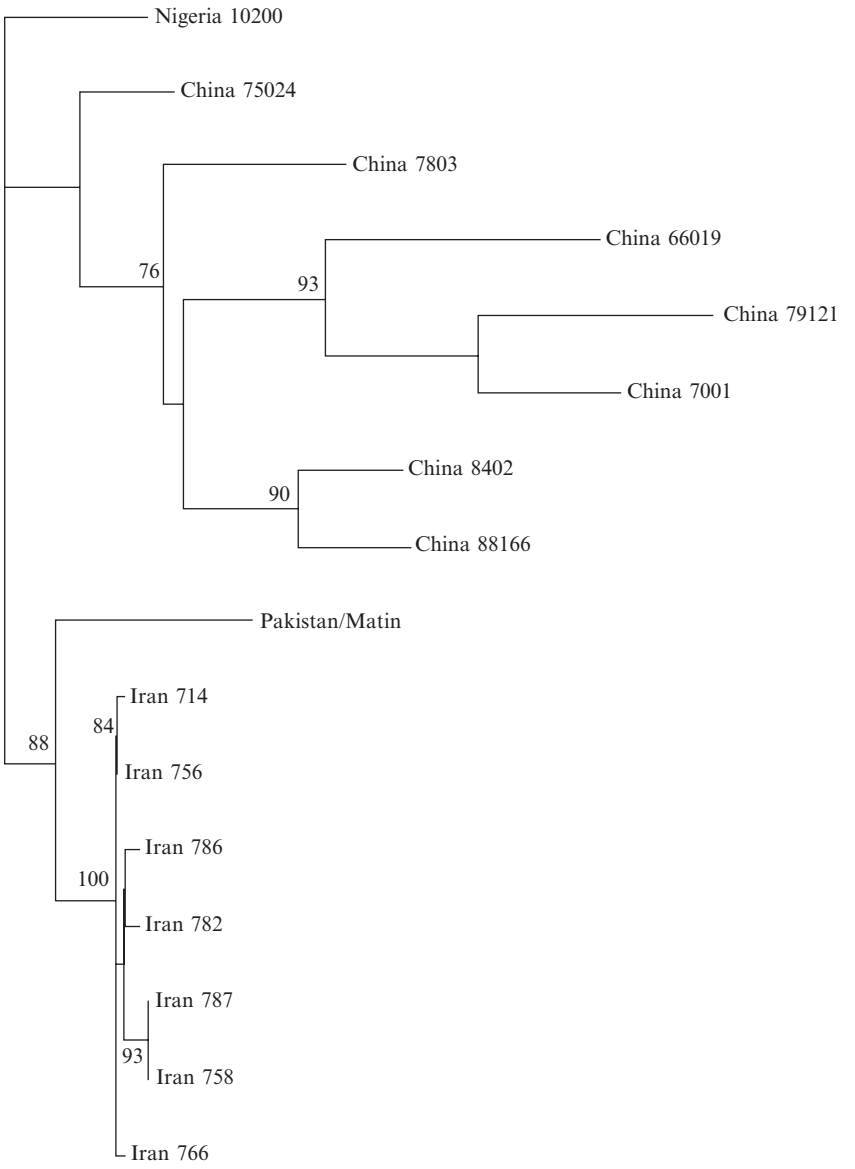

Fig. 8-3. Phylogenetic tree (neighbor-joining) calculated for Iranian CCHFV M-segment sequences.

ribavirin are inaccessible). According to the protocol of the CDC of Iran, the dose of ribavirin are recommended as follows:

- First 30 mg of ribavirin/kg of body weight
- Then 15 mg of ribavirin/kg of body weight each 6 h for 4 days
- At the end 7.5 mg of ribavirin/kg of body weight each 8 h for 6 days
- Also patients receive supportive therapy [11, 12, 26]

8.9. PREVENTION

The prevention measures performed in Iran are based on two axes: (1) public education and (2) ticks control. Concerning public education, seminars and speeches have been organized in collaboration with the Pasteur Institute of Iran and Ministry of Health, CDC of Iran, in the at-risk provinces of the country in the universities and health centers.

For the common people, mass media and several press articles have exposed the ways and risks of transmission CCHF and explained in detail the prevention measures and the ways to control it.

Concerning ticks control, all livestock are treated in industrial breeding center with insecticide showers, also in industrial slaughterhouses, persons are educated about the different ways of the transmission and the control of the disease, as slaughterers wear gloves, goggles, plastic gowns, and other preventive things, so the contamination risk is reduced.

In the traditional way of breeding livestock, there is less controls and preventive measures and livestock are infected with ticks carrying CCHF virus and there is more chance to contaminate oneself when slaughter these animals or handling their carcass and meat.

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