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Seroprevalence, Incidence and Risk Factors of Crimean-Congo Hemorrhagic Fever in Sistan-va-Baluchestan Province, Iran

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Abstract
Since 1999, many cases of Crimean-Congo Hemorrhagic Fever have been reported from different parts of Iran. This study intended to define CCHF seroprevalence, incidence rates, and the most important risks in Sistan- Baluchestan province, Iran. Using cluster sampling with probability proportional to size, 310 subjects were selected from various districts of Zahedan and Zabol in the northern part of the province. Blood samples were drawn from consenting subjects, once at the beginning of the study and then 6 months later. The study began on 22 April 2003 and continued till 17 February 2004. A total of 18 out of 285 subjects who consented to give blood samples were actually positive by IgM and IgG capture ELISA tests. The calculated seroprevalence was 6.32% (95% CI: 3.24% to 9.40%). The calculated incidence was 0.48% (95% CI: 0.00% to 1.44%). Fourteen out of the 18 subjects with definitely positive IgG or IgM were female. In multivariate analysis, age, education, and history of slaughtering livestock were significantly related to the risk of infection. Only one of the seropositive subjects had a history of tick bite. This study shows the importance of subclinical infections in the epidemiology of this disease in Iran. It also seems that the risk of infection for housewives is high. Regardless of the high female to male ratio of seropositive subjects, it appears that the epidemiologic characteristics of the infection in this region are not so different compared to other parts of the world.

Keywords: Crimean-Congo Hemorrhagic Fever, Sistan-va-Baluchestan, Infection disease, Iran

Introduction
The causative agent of Crimean Congo Hemorrhagic Fever is a virus of the family Bunyaviridae, *Nairovirus* genus. The earliest cases were reported from the Crimean peninsula in 1944 and later in 1969 from Congo. All viral hemorrhagic fevers are zoonoses. The typical route of transmission in nature is through tick bite. Ticks of the genus *Hyalomma* are the main vectors, and reservoir of the virus in the nature (1). These ticks transmit the virus transovarially to the next generation (2, 3). In mammals (domestic animals, rodents) and ground-feeding birds the infection is usually subclinical and lasts from a few days to a few weeks (4 - 8).
Epidemiologic studies in Africa show that disease propagation in tropical and subtropical areas occurs more easily than in other areas (8, 9). Within the past few years there have been many reports of CCHF in countries neighboring Iran (10 - 15).
Since 1969, there have been reports of a disease

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with hemorrhagic manifestations (very similar to the clinical picture of "hemorrhagic fevers") in northwestern Iran. In 1975 a large seroprevalence study was performed in the northern half of the country. In this study 13% of human sera and 38% of sheep sera were positive for CCHF (16). The human sera were obtained from apparently healthy individuals in rural areas with high exposure to tick vectors.

Afterwards there were no reports or studies of CCHF in Iran until 1999, when a hospital outbreak was reported form Shahr-e-Kord district in central Iran. Following that report, other outbreaks were recorded - especially in regions trading livestock with Sistan-va-Baluchestan Province. Many studies about CCHF were performed during these outbreaks; one of the most important of these studies was the one about efficacy of oral ribavirin in the treatment of CCHF (17). So far the highest incidence rates have come from the northern parts of Sistan-va-Baluchestan province (Zabol and Zahedan districts) (18-20). The numbers of confirmed cases from this province have been several times higher compared to the other areas in Iran.

Hyalomma ticks and particularly *H. Marginatum*, the main natural reservoir and vector of CCHF, are common all over Iran. The heaviest infestation is found in sheep and goats and the lowest in horses and cattle (16, 21).

The number of confirmed CCHF patients with a positive history of tick bite is strangely low. Most cases present without any history of recent tick bite.

The main objectives of this study were:

1) Determining the epidemiological characteristics of CCHF in Sistan-Baluchestan province, focusing on CCHF seroprevalence;
2) Studying the different modes of transmission and determinants of CCHF infection;
3) Estimating the incidence of the disease in Sistan-va-Baluchestan province, Iran.

Materials and Methods

The study population was composed of the 800,000 residents of Zahedan and Zabol districts in the northern part of Sistan-va-Baluchestan. The study area, which is in the northern half of the province, is in neighborhood of Afghanistan and Pakistan from east.

To determine the seroprevalence, IgM and IgG capture ELISA tests were used. IgM is detectable mainly within the first 4 months of onset and is used for detection and /or confirmation of ongoing or recent infection. While, IgG remains detectable for at least 5 years after infection (22).

During interview with every individual a questionnaire containing questions about demographic characteristics and the determinants of infection during the preceding 4 months was completed and at the end a blood sample was taken. Blood samples were transferred to a local laboratory where sera were extracted and frozen. The frozen sera were transferred to the Pasteur Institute of Iran (under cold-chain regulations and by airplane) for IgG capture ELISA and IgM capture ELISA tests. All lab tests were done in blind circumstances (i.e. the lab technicians did not know anything about the subjects).

There were no age or geographical restrictions in selecting the study subjects and the sampling method was a modified form of the classical probability-proportional-to-size (PPS) cluster sampling (23).

The original cross sectional study was followed by a cohort study of the portion of the original study population still at risk of developing the disease (i.e. seronegative subjects). Seronegative controls identified during the cross sectional phase were subjected to follow-up over a 6-month period for the development of clinical signs and symptoms of the disease. These were further tested by serological tests. Therefore, the study design was of the survey follow-up type, a hybrid design combining the elements of

the two basic designs; both cross sectional and prospective cohort designs back to back.

To study the determinants and risk factors of infection, a case-control study was conducted based on the results of the cross-sectional phase. Subjects having a positive IgM or IgG capture ELISA (with or without clinical history) were selected as cases and seronegative subjects (those with negative IgM and IgG ELISA) without a clinical history of hemorrhagic fever served as controls.

Results

The first round of blood sampling began on 22 April 2003 and ended on 28 July 2003. During this period 285 of 310 subjects who were interviewed were consent for blood sampling. In the first round of blood sampling, 14 of the 285 blood samples were positive on ELISA tests for anti-CCHF IgG and IgM. Eight subjects were IgM positive while 7 were IgG positive. One of the subjects was both IgG and IgM positive. In addition, 3 subjects were borderline-positive for IgM. The second round began on 28 January 2003 and ended up on 17 February 2004. In the second round all of these 17 subjects were seropositive for IgG. In addition, one of the subjects who were seronegative in the first round turned IgG-positive in the second round. Therefore the number of seropositive subjects reached 18 by the end of the study. The mean time period of follow up was 225.2 d (SD = 1.18 d).

Table 1 shows the most important characteristics of the subjects who consented to blood sampling (for some of the variables the total does not come to 285; this is due to incomplete responses in some of the questionnaires). Considering all of the 18 cases, the seroprevalence of the CCHF infection in the study population would be 6.32% (95% CI: 3.24% to 9.40%).

Of those subjects who were sampled for the second time (226 people), only one had experienced seroconversion. The calculated incidence rate was 0.48 (95% CI: 0.00 to 1.44). Calculation of incidence based on the person-years of follow-up was 0.74% (95% CI: 0.00 to 2.21).

In crude analysis sex (P= 0.048, one sided exact test), age (P = 0.032, linear trend), education level (P = 0.045, linear trend), and job (P= 0.037, chi-square) show significant relationship with disease.

In adjusted analysis (using logistic regression), age, education level and, history of slaughtering livestock were significantly related to the chance of infection. Table 2 shows the results of adjusted analysis.

Four of the 310 interviewed subjects reported a history of tick bite within the past 4 months (one in the case group and the others in the control group). Only 2 of the interviewed subjects had a history of contact with a CCHF case and none of them were seropositive.

Of the 285 subjects sampled in the first round, 249 were successfully followed up in the second round. Of these, 22 (8.8%) had a history of febrile disease between the first and second visits. Five patients (2%) had signs and symptoms compatible with CCHF (epistaxis, gum and/or rectal bleeding and history of contact with livestock). One of these febrile subjects refused to give a sample in the second round. However serum samples from the remaining febrile patients (i.e. 21 samples) were tested in the Pasteur Institute of Iran using IgG capture ELISA. None of these 21 febrile subjects were positive by ELISA.

Table 1: Characteristics of subjects consenting to blood sampling. (Seropositives are all ELISA IgG and IgM positive subjects)

Parameters	Seronegative No. (%)	Seropositive No. (%)
District of residence		
Zahedan	148 (55.4)	10 (55.6)
Zabol	119 (44.6)	8 (44.4)
Nationality		
Iranian	209 (80.7)	12 (66.7)
Afghan	50 (19.3)	6 (33.3)
Living environment		
Urban	113 (43.0)	5 (27.8)
Urban with Rural culture	54 (20.5)	4 (22.2)
Rural	96 (36.5)	9 (50.0)
Age groups		
0 – 20 years	141 (53.2)	6 (33.3)
21 – 40 years	87 (32.8)	6 (33.3)
41 – 60 years	31 (11.7)	5 (27.8)
≥ 60 years	6 (2.3)	1 (5.6)
Sex		
Male	120 (44.9)	4 (22.2)
Female	147 (55.1)	14 (77.8)
Education		
Illiterate	101 (39.3)	11 (64.7)
1 – 5	61 (23.7)	3 (17.6)
≥ 6	95 (37.0)	3 (17.6)
Job groups		
High risk groups(1)	21 (7.9)	4 (22.2)
Low risk groups(2)	246 (92.1)	14 (77.8)
History of contact with Livestock		
Never	90 (33.3)	3 (16.7)
Infrequently	93 (34.4)	6 (42.9)
Frequently	87 (32.2)	5 (35.7)
History of slaughtering of Livestock		
1) Never	124 (47.1)	5 (29.4)
2) Only in Religious ceremony	76 (28.9)	5 (29.4)
3) Usually but not professionally	59 (22.4)	6 (35.3)
4) Professionally	4 (1.5)	1 (5.9)
Presence of livestock within the past 4 months		
Yes	133 (49.6%)	7 (50.0%)
No	135 (50.4%)	7 (50.0%)

1) Shepherd, farmer, husbandry worker, butcher, slaughterhouse worker, veterinarian, physician, nurse, health worker

2) Housewives, children (under 12 years), soldiers, workers, others.

Table 2: Results of adjusted analysis (logistic regression)

Variables	Odds ratio	95% CI	P-value (Likelihood ratio test)
Age (year)	1.03	1.00-1.05	0.033
Education level (year)	0.92	0.80-1.05	0.002
History of slaughtering 1)	1.82	0.64-5.25	0.004

Groups 1&2 versus 3&4 (from Table 1)

Discussion

Between the two phases of the study, seroconversion occurred in only one of the subjects. This was a 33-year-old male living in rural Zabol. He is an illiterate Afghan shepherd. All the subjects who were borderline-IgM positive became IgG positive in the second phase of the study.

As mentioned in the results section, the seroprevalence of anti-CCHF antibodies was 6.32%. In calculating the confidence interval, (95% CI: 3.24% to 9.40%) the design effect of the cluster sampling has been considered (23). In another study with a similar design performed about 16 months earlier in the same area and with a similar sample size (300 subjects), estimated seroprevalence was 2.4% (95% CI: 0.3% to 4.4%). This figure was calculated on the basis of IgG capture ELISA tests (24).

One of the most striking features of the seropositive group is that 14 out of 18 cases (77.7%) were females (female/male = 6/1). This finding is contrary to the notion of an occupational infection, which would be expected to occur mainly in men. It is possible that the high rate of infection in females and in the urban areas is handling of the meat less than one hour after slaughtering both in rural and urban areas.

In the other studies, female-to-male ratio did not exceed one. In a study of clinically diagnosed CCHF cases by the Sistan-Baluchestan Province Health Center, 42% of the cases were

female (15). In another report from the "Iranian Center for Disease Control and Prevention" 40.7% of the serologically confirmed IgG and IgM cases were female (18). In a study on a nomadic population in Senegal, 59.5% of the IgG positive subjects were female (25). However, none of these studies showed a statistically significant relationship between sex and risk of infection.

In our study the risk of infection was shown to increase with age (P= 0.027). This is in accordance with the other studies in Senegal (P= 0.001) and South Africa (P<0.001) (25, 26). The finding is not surprising, as with increasing age there is greater chance of having an effective exposure.

In multivariate analysis education level shows an inverse relationship with the risk of infection. In fact the risk is higher for those with primary-level education and below. This is hardly surprising, considering the relationship between education and health-related behaviors.

In multivariate analysis, a history of slaughtering livestock is another variable associated with the risk of infection (P= 0.004). In other words, slaughtering livestock as a profession increases the risk of infection. Slaughtering is a well-known risk factor for CCHF and these findings agree with results of studies in other parts of the world. In this regard, the epidemic in an ostrich abattoir in South Africa is particularly notable (27). In another study in South Africa, hunting and contact with the carcasses of wild animals also increased the risk of infection (26).

Only 4 of the 310 interviewed subjects had a history of tick bite within the past 4 months and only one of them was seropositive. However in another population-based case control study using hospitalized CCHF cases, 8 of 22 cases reported a history of tick bite and such bites was one of the most important risk factors for the disease (adjusted odds ratio=106, P=0.000) (28). The obvious difference between subclinical and clinical cases with respect to the history of tick bite may be due to the effect of the mode of transmission on disease virulence. In other words it seems that pathogenicity and virulence is lower with modes of transmission other than tick bite.

The number of subjects who had a history of contact with CCHF cases (only 2 seronegative subjects) was insufficient for a conclusive analysis. However, there is another well-designed investigation going on about the infectivity of these patients.

In the present study none of the seropositive subjects had any history of clinical CCHF. In the first round of blood sampling some of the subjects were positive only for IgM, in other words they had to be in the acute phase of infection at the time of sampling, without showing any signs or symptoms. These findings underline the importance of subclinical cases in the epidemiology of this disease. The role of such cases in transmission of infection is yet to be defined. This study shows a high degree of risk for housewives and the finding is important in planning future prevention schemes. The study also marks a notable departure from previous views on high-risk groups (mostly occupational). Other findings, including increased risk with age, slaughtering and low-level education are in accordance with findings in other countries.

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