



## Seroepidemiology and risk factors of Crimean-Congo Hemorrhagic Fever among butchers and slaughterhouse workers in southeastern Iran



Ehsan Mostafavi<sup>a,\*</sup>, Behzad Pourhossein<sup>a,b</sup>, Saber Esmaili<sup>a,c</sup>, Fahimeh Bagheri Amiri<sup>d</sup>, Sahar Khakifrouz<sup>e</sup>, Nariman Shah-Hosseini<sup>e</sup>, Seyyed Mehdi Tabatabaei<sup>f</sup>

<sup>a</sup> Department of Epidemiology and Biostatistics, Research Centre for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran, Tehran, Iran

<sup>b</sup> Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

<sup>c</sup> Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

<sup>d</sup> Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>e</sup> Department of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory), Research Centre for Emerging and Reemerging Infectious Diseases, Pasteur Institute of Iran, Tehran, Iran

<sup>f</sup> Infectious Disease and Tropical Diseases Research Center, Zahedan University of Medical Sciences, Boo-Ali Hospital, Zahedan, Iran

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### ABSTRACT

**Objective:** Crimean-Congo Haemorrhagic Fever (CCHF) is a viral zoonotic disease. Butchers and slaughterhouse workers are considered to be high risk occupational groups for the disease. Sistan and Baluchistan province is an area in southeastern Iran which is endemic for CCHF, and the most confirmed cases of the disease are reported from this province. The aim of this study was to investigate the seroprevalence of CCHF and risk factors for seropositivity among them in Sistan and Baluchistan province in 2011.

**Methods:** Questionnaire data and blood sample collection were carried out for each participant and the sera samples were sent to the national reference laboratory for ELISA IgG testing.

**Results:** In this study, the seroprevalence of CCHF among 190 butchers and slaughterhouse workers from 11 counties was 16.49%. 79% of participants were aware that they were at risk of zoonosis and 39.7% did not use any personal protective equipment during their work. Of 31 CCHF IgG positive individuals in this study, eleven individuals had a previous record of CCHF infection in 57 months prior to the study.

**Conclusions:** High seroprevalence of CCHF among butchers and slaughterhouse workers and minimal use of personal protective equipment's during daily work indicates the need for training courses, for these groups to increase their knowledge, attitude and practice with respect to zoonosis.

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### Introduction

Crimean-Congo Haemorrhagic Fever (CCHF) is a zoonosis caused by a virus belonging to the genus Nairovirus, Bunyaviridae family (Whitehouse, 2004). CCHF was first described in 1944 in the Former Soviet Union and is now considered to be an important disease in Eastern Europe, Africa and Asia (Casals, 1969; Swanepoel et al., 1989). Slaughterhouse workers and butchers, farmers and ranchers, veterinarians and health care workers are considered

high risk occupational groups (Izadi et al., 2004; Whitehouse, 2004). Contact with infected animals or their blood, secretions and carcasses, and tick bites are the most common mode of virus transmission to humans (Fisgin et al., 2009; Ozturk et al., 2012). The clinical symptoms of CCHF include fever, headache, myalgia, nausea and bleeding from body cavities (Swanepoel et al., 1989). Increased ALT and AST levels and elongated PTT with leucopenia or leukocytosis are the main laboratory and haematological findings of CCHF (Chinikar et al., 2008; Ozkurt et al., 2006). The mortality rate of the disease varies between 5–80% (Whitehouse, 2004; Williams et al., 2000).

After human infection, antibodies will be produced against different components of the virus such as surface glycoproteins (Gn, Gc), viral nucleoproteins and genomic fragments, and the dominant antigen will be viral nucleoproteins, the recombinant form of which is mainly used in CCHF diagnoses and antibody

\* Corresponding author at: No. 69, Pasteur Ave., Department of Epidemiology and Biostatistics, Research Centre for Emerging and Reemerging infectious diseases, Pasteur Institute of Iran, Postal Code: 1316943551, Tehran, Iran. Fax: +98 21 66496448.

E-mail address: [mostafavi@pasteur.ac.ir](mailto:mostafavi@pasteur.ac.ir) (E. Mostafavi).

titration (Garcia et al., 2006). Different methods such as Indirect Fluorescent Antibody (IFA) and Enzyme-Linked Immunosorbent Assay (ELISA) are used as serological methods for diagnosis (Ergonul, 2006). IgM antibodies are the first class of antibodies to appear in blood 7–10 days after initial infection. IgM reaches its peak in two or three weeks and will disappear in the fourth month (Shepherd et al., 1989). About 2–4 months following infection, IgG antibody titre reaches its highest level, its presence having been studied for 5 years (Shepherd et al., 1989). IgG detection is therefore appropriate for epidemiological surveys.

Sistan and Baluchistan province is an area in south-eastern Iran which is endemic for CCHF, (Sharifi Mood et al., 2008) and the most confirmed cases of the disease are reported from this province. The most CCHF cases in this province are reported among butchers and slaughterhouse workers (Chinikar et al., 2005). This study was performed to survey CCHF seroprevalence and its risk factors among butchers and slaughterhouse workers in Sistan and Baluchistan province. In addition, as some of the participants in this study had a history of CCHF infection, the length of IgG existence was studied in these people.

## Materials and methods

### Study area

This study was carried out in Sistan and Baluchistan province in south-eastern Iran in 2011. The province consists of two regions, Sistan in the north and Baluchistan in the south. Sistan and Baluchistan province is currently one of the driest regions of Iran with a slight increase in rainfall from east to west and an obvious rise in humidity in the coastal regions. This province is the largest province in Iran (with an area of approximately 187,502 km<sup>2</sup>) and according to a 2011 report has a population of 2,534,327. Sistan and Baluchistan province is bordered by the Oman Sea to the South, Afghanistan and Pakistan to the East, South Khorasan province to the north and Kerman and Hormozgan provinces to the west.

### Ethical considerations

The ethical committee of the Pasteur Institute of Iran approved the consent procedure, the proposal and protocol of this study, covering all the samples taken (blood) and questionnaire. Verbal informed consent was obtained as most of the participants were either illiterate or had a primary education. All human subjects were adult.

### Sampling process

Sampling was performed randomly from butchers and slaughterhouse workers in all counties (Zahak and Zabol in the north, Zahedan, Iranshahr and Khash in the centre and Saravan, Sarbaz, NikShahr, Konarak and Chabahar in the south) of Sistan and Baluchistan province. Participants were  $\geq 18$  years old with a minimum of 6 months' work experience. After obtaining informed consent, information was collected from each participant, such as demographic characteristics, exposure to risk factors during work, usage of personal protective equipment, and their knowledge and attitude regarding zoonotic diseases, by means of a researcher-made questionnaire. After completion of the questionnaire, blood samples were collected from each participant and the serum was separated. Sera were kept at  $-20^{\circ}\text{C}$  and transferred to the Laboratory of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory) at the Pasteur Institute of Iran (Tehran).

### CCHF IgG detection

Serum samples were analyzed by specific ELISA for IgG. IgG detection involved coating the ELISA plates with mouse hyperimmune ascetic fluid (diluted at 1:1000) in phosphate-buffered saline (PBS 1x) and incubating overnight at  $4^{\circ}\text{C}$ . Following the washing step, the recombinant antigen (diluted at 1:500) in PBS containing 0.5% Tween (PBST) and 3% skimmed milk (PBSTM) was added, and the plates were incubated for 3 h at  $37^{\circ}\text{C}$ . Serum diluted at 1:100 in PBSTM was added and the plates were incubated for 1 h at  $37^{\circ}\text{C}$ . Peroxidase-labeled anti-animal immunoglobulin diluted at 1:1000 in PBSTM was added and the plates were incubated for 1 h at  $37^{\circ}\text{C}$ . The plates were washed 3 times with PBST after each incubation. Finally, 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 4 N H<sub>2</sub>SO<sub>4</sub>. The plates were read by the ELISA reader (Anathos 2020) at 450 nm.

### Statistical analysis

The data were analysed by SPSS software (version 16, SPSS Inc, Chicago). Chi-square, Fisher's exact and regression logistic tests were used to compare the variables during analysis. P-values less than 0.05 were considered statistically significant, and P-values between 0.05 and 0.1 were considered weakly significant. Six questions (use of masks, boots, overalls and gloves, and disinfecting equipment and hands and face after work) with five conditions (always, often, sometimes, seldom or never) were asked of each participant to check the status of the use of protective equipment and performance of the hygiene factors. Total scores of each participant were assessed, and compared to the median scores of all participants.

## Results

One hundred and ninety samples were collected from butchers and slaughterhouse workers from 11 counties of Sistan and Baluchistan province. The median (maximum, minimum) age and length of employment of participants in this study were 33.5 (18, 86) and 8 (1, 44) years, respectively. All participants were male. 96.8% of participants were satisfied with their current job and 79.9% were aware that they were at risk of zoonosis.

Overall, 162 (85.3%) participants were involved in the slaughtering of animals, 43 (22.6%) in the handling of animal residue, and 2 (1.1%) were merely involved in meat inspection during their daily work. 161 (84.7%) participants were in contact with sheep and goats, 143 (75.3%) with calves and cows, and 80 (42.1%) with camels, as part of their daily activities. 75.3% of workers had a history of being splashed with fluids of animals viscera for more than once on their faces and 80% on other parts of their bodies. 25.8% of these individuals had a history of cutting their hands or other parts of their bodies at least once during their work and 17.4% recalled an ectoparasite bite during the last year. 39.7% of participants did not use any personal protective equipments (mask, gloves, overalls or boots), while 22.8% always used them. 83.6% of participants had never applied chemical disinfectant to disinfect their knives, hands and faces.

CCHF seroprevalence among the participants was 16.49%.

A total of 16 (8.42%) participants mentioned that they had been infected with CCHF or a similar haemorrhagic disease, of which 11 persons had been serologically confirmed as being positive for CCHF infection by the National Reference Laboratory data bank. These participants had been infected by CCHF 57.09 months (standard error = 12.71) ago. Our data illustrated that one sample with an initial infection 10.5 years ago (126 months) and two

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