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

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 leucopoenia 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

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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 Baluchestan province in south-eastern Iran in 2011. The province consists of two regions, Sistan in the north and Baluchestan in the south. Sistan and Baluchestan 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^2) and according to a 2011 report has a population of 2,534,327. Sistan and Baluchestan 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 Baluchestan 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 °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 °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 °C. Serum diluted at 1:100 in PBSTM was added and the plates were incubated for 1 h at 37 °C. Peroxidase-labeled anti-animal immunoglobulin diluted at 1:1000 in PBSTM was added and the plates were incubated for 1 h at 37 °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_2SO_4. 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

samples with an initial infection 9 years ago (108 and 113 months ago) were still seropositive (IgG) (Table 1).

There was a significant difference in CCHF seropositivity across the different sampling regions (p = 0.04). The Northern areas of the province (25.6%) and Zahak county (in the north) (60%) had the highest CCHF seroprevalence. CCHF seropositivity in the north region was 7.56 times (OR: 7.56, 95% CI: 1.56, 36.49) higher than the southern region of the province, and CCHF seropositivity in the central region was 4.89 times (OR: 4.89, 95% CI: 1.08, 22.05) higher than the south region of Sistan and Baluchestan province. There was no significant difference between the Northern and central regions (p = 0.32) (Table 2).

Individuals with a longer length of employment had significantly higher levels of seropositivity than others, and there was a poor positive correlation between age and CCHF seropositivity (p = 0.08). Other risk factors such as livestock slaughtering, handling of animal residue, splashing animal secretions to the body or face, cutting of the hand, ectoparasite bites and the type of livestock slaughtered, had no effect on seropositivity (Table 3).

Table 2
Seroprevalence of CCHF among butchers and slaughter workers in Sistan and Baluchestan province according to region and county.

Region/Counties	No. tested	% seroprevalence (95% CI)
North	43	25.58 (14.25–40.11)
Zahak	5	60.00 (18.24–92.65)
Zabol	38	21.05 (10.29–36.09)
Centre	99	18.18 (11.50–26.70)
Iranshahr	14	0.00 (0–19.26)
Zahedan	73	21.92 (13.55–32.48)
Khash	12	16.66 (2.89–45.06)
South	46	4.35 (0.73–13.63))
Chabahar	10	10.00 (0.50–40.35)
Sarbaz	6	0.00 (0–39.30)
Konarak	10	0.00 (0–25.89)
Saravan	10	10.00 (0.50–40.35)
NikShahr	10	0.00 (0–25.89)
Total	188	16.49 (11.69–22.31)

Discussion

In this study CCHF seroprevalence was 16.49% among butchers and slaughterhouse workers of Sistan and Baluchestan province in south-eastern Iran. This rate was much higher than CCHF seroprevalence among the general population in this province in comparison with another study (2.4%) (Izadi et al., 2006); given this difference, it is evident that butchers and slaughterhouse workers are among high risk occupations for CCHF. A comparison between the present study and other similar studies in Iran show that the seroprevalence of CCHF is higher in butchers and slaughterhouse workers of Sistan and Baluchestan province. CCHF seroprevalence in these occupational groups has been reported at 5% in Isfahan province (central Iran) (Karimi et al., 2007), at 7.4% in Yasuj city (southwest Iran) (Hadinia et al., 2012) and at 14.8% in north-eastern Iran (North Khorasan, Razavi Khorasan and south Khorasan provinces) (Chinikar et al., 2012). In a survey in Turkey, CCHF seroprevalence among people with a history of livestock slaughtering was 16.6% (Gunes et al., 2009), which was almost similar to the present study.

In the present study, CCHF seroprevalence was higher in the northern regions when compared to the southern and central regions. It may be because of this fact that animal husbandry is more common in the northern region as compared with the south. It should be noted that the majority of clinical patients are reported from the Northern region of this province (Zahak, Zabol and Zahedan counties), which is due to the higher population in the Northern region of the province (Izadi et al., 2006; Naieni et al., 2004). Moreover, animal husbandry is more common in the northern region as compared with the south. The results of the present study showed that in addition to high population density in the northern area, slaughtered livestock in the Northern region probably have a high level of infection with CCHF, which causes a higher level of seropositivity in butchers and slaughterhouse workers in this region.

In the present study, length of employment and age had a positive correlation with CCHF seropositivity. It seems logical to suggest that as length of employment and age increase, the chance and rate of direct or indirect contact by pathogen also increase and this finding is in parallel with other studies (Chapman et al., 1991; Naieni et al., 2004; Wilson et al., 1990).

Sixteen participants had a history of CCHF or a similar infection; data relating to 11 of them existed in the National Reference Laboratory of Arboviruses and Viral Haemorrhagic Fever serum bank, and all of them were IgG seropositive in the present study. Five other participants who had no records in the national reference laboratory and were IgG sero-negative in this study were probably infected with other diseases.

In the present study, the average length of IgG seropositivity was approximately 5 years (57.09 months). This finding is comparable with two other studies which have reported CCHF IgG three and five years following infection (Burt et al., 1994; Shepherd et al., 1989).

Interestingly, three samples belonging to participants with a CCHF infection history dating back to 9 years ago were still IgG positive. There are two probabilities to explain these cases: IgG remained detectable in these persons following the first infection,

Table 1
Characteristics of CCHF IgG positive butchers and slaughterhouse workers in Sistan and Baluchestan province, south-eastern Iran, with a history of previous CCHF infection.

Counties	Date of previous CCHF infection	Duration (month) of CCHF infection until sampling time (November, 2011)	Age (year)	Length of Employment (year)
Zahedan	04/11/2010	19	38	2
	06/01/2001	126	25	2
	06/30/2002	113	28	10
	10/24/2009	25	29	10
Zabol	03/17/2006	68	44	17
	07/24/2008	40	62	30
	10/30/2010	13	29	7
	11/10/2002	108	33	8
	12/01/2010	12	38	8
Zahak	05/14/2006	66	40	8
Khash	09/13/2008	38	23	7

Table 3
Analysis of risk factors associated with CCHF among butchers and slaughterhouse workers in south-eastern Iran.

Risk factor	No. tested (% infected)		OR (95% CI)	p-Value
	Positive exposure	Negative exposure		
Age[a]	93 (21.51)	93 (11.83)	2.04 (0.92,4.55)	0.08
Length of Employment[b]	96 (21.88)	84 (10.71)	2.33 (1.00,5.43)	0.04
Slaughtering	161 (18.01)	14 (7.14)	2.86 (0.36,22.71)	0.47
Transportation of the remaining livestock	43 (19.95)	132 (18.18)	0.73 (0.28,1.92)	0.52
Animal secretions to Face	47 (12.77)	141 (17.73)	0.68 (0.26,1.77)	0.43
Animal secretions to body	36 (16.66)	151 (16.56)	1.01 (0.38,2.68)	0.99
Occupational injury				
Cutting hand or other parts of body[c]	49 (22.45)	134 (14.93)	1.65 (0.73,3.76)	0.23
Ectoparasite bite[d]	9 (11.11)	179 (16.76)	0.62 (0.08,5.15)	0.55
Contact with animals				
Cattle	141 (14.89)	45 (20.00)	0.70 (0.29,1.66)	0.42
Sheep & Goats	160 (15.00)	26 (23.07)	0.59 (0.21,1.62)	0.39
Camels	80 (17.50)	106 (15.09)	1.91 (0.55,2.62)	0.66
All 3 groups of animals	73 (16.43)	113 (15.93)	1.04 (0.47,2.31)	0.93
Practice (personal protection) and attitude				
Personal Protection[e]	95 (13.68)	93 (19.35)	0.66 (0.30,1.44)	0.30
Considering self at risk of zoonosis diseases	149 (18.12)	38 (10.53)	1.88 (0.62,5.75)	0.26

[a] Age less than 33.5 years (median age of participants) was considered as negative exposure.
[b] Work experience of less than 8 years (median length of employment of participants) was considered as negative exposure.
[c] Cutting hand or other body part during work less than 5 times (median point of participants) was considered as negative exposure.
[d] Bite by ectoparasite during work less than 5 times (median point of participants) was considered as negative exposure.
[e] Less than 12 points (middle point of participants) was considered as negative exposure.

or these persons were once again exposed to CCHFV during the following years, which enabled the antibody titre to be detected again in this study.

One of the limitations of this study was the absence of a control group as representative of the general population in order to obtain a better risk assessment in the studied group; we suggest that this aspect can be considered in similar subsequent studies.

Conclusion

According to the high level of seroprevalence among butchers and slaughterhouse workers in the present study, it is suggested that these occupations should be monitored in other areas of the country. On the other hand, due to the lack of personal protective equipment, holding special training classes or the publishing of health information leaflets by butcher trade officials is required to persuade and inform these groups to use personal protection equipment.

Conflict of interest

The authors declare that they have no conflict of interest.

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Ethical approval

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), questionnaire and verbal informed consent as most of the participants were either illiterate or had a primary education. All human subjects were adult.

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