SEROEPIDEMIOLOGY OF CRIMEAN-CONGO HAEMORRHAGIC FEVER IN RURAL COMMUNITY OF BASRAH

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ABSTRACT

A seroepidemiologic survey of Crimean-congo haemorrhagic fever (CCHF) was carried out on rural community of Basrah, southern Iraq from November 1st 1996 through June 1st 1997. A total of 682 serum samples were obtained from apparently healthy individuals with their age range from 5 to 76 years and nearly 20% of these sera were obtained from occupational risk group (veterinarian, abattoir workers, and farmers). Serum samples from domestic animals were collected from areas where CCHF cases were recognized. A total of 74 sheeps and 48 cattle sera were collected parallel to the collection of 42 tick's pools from these animals. An enzyme-linked immunosorbent assay (ELISA) was used for the detection of antibody against CCHF virus in human and animal sera. The prevalence of IgG antibody to CCHF in residents of rural community of Basrah was 4.3% indicating that CCHF virus is circulating below the endemic level. Seropositivity to CCHF in northern Basrah inhabitants was 9.7% while seropositivity in animals was observed in 20% of sheeps and 37% of cattles in this area. Ticks identification revealed that 100% of the identified ticks from northern Basrah were adult ticks of Hyaloma marginatum. The ecologic and economic characteristics of the area are the major variables that assisting the existence of enzootic focus for CCHF virus in northern Basrah.

INTRODUCTION

Arboviruses are large and taxonomically diverse group of zoonotic viruses, transmitted by blood sucking arthropoda vector.[1,2] Infections ranged from a flu-like disease (dengue and papatasi) to a severe but not fatal viral syndrome 'encephalitis'(EEE, WEE, CAE) or a rapidly progressive and frequently fatal haemorrhagic fever (Rift valley, CCHF).[3,4] For the past 50 years arboviruses were demonstrating an emergence of the pandemic interest, particularly after the striking and explosive enzootic of Rift valley fever in lower Nile delta in Egypt 1977 in which an estimated 200,000 cases with 598 deaths was recorded.[5-8] The first recognized outbreak of CCHF in Iraq was in 1979 with nosocomial infection which has led to the death of two medical staff as a result of contact with index case.[9] From 1979 to 1980 sixty cases of CCHF have been reported with 41 deaths (70% fatality rate) and 100% aborted pregnant female.[10] Following 1979 outbreak, clinical, virological and serological surveys were conducted to assess the extent of the epidemic areas in Iraq and the source of the index case.[11,12]

The aim of this study is to explore the extent of the enzootic focus of CCHF in rural community of Basrah by following the distreibution of the virus main vector, the tick of *H.marginatum* parallel with the investigation of antibody prevalence to CCHF virus in human and animal sera.

MATERIAL AND METHODS

Sampling system: Sera collection was organized to meet the epidemiologic objectives of the study. Basrah rural areas were grouped into three zones, designated A (Qurna, Medaina, Hartha: where Basrah CCHF index case was recognized 1985), zone B (Zubair, Safwan, Lehais: where the 1993 CCHF case was reported) and zone C (Fao, Abu-Alkhasib, Shatt-Alarab and Al-Faiha). Sersa were collected at each area from health centers included apparently healthy individuals and from occupational risk groups who were in contact with animals. A total of 236 serum samples were collected from area A; 178(94 males and 84 females) at HC and 58 occupational samples (44 males and 14 females), 201 serum samples were collected from area B (158 from HC: 97 males and 61 females) and 43 occupational samples (32 males and 11 females) and 190 samples from area C (150 from HC: 85 males and 65 females) and 40

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occupational samples (28 males and 12 females). The occupational group includes: veterinarians of official and private veterinary clinics, abattoir workers and farmers who were in contact with animals. For comparison and control 55 serum samples were collected: 32 persons subjected to AIDS screening programe at public health laboratory(PHL) who had never been in contact with animals and 23 samples from long contact group, giving an overall sample collection of 682 (423 males and 259 females). Sera were also collected from domestic animals around places where CCHF cases were reported. Sera from 74 sheep and 48 cattles beside 42 tick's pools were collected from infested animals (Tick pool: 10 or more ticks from one animal kept in one vial) following Hoogstreal et al 1981 method.[13] Serum samples were collected from close contact of fatal case of CCHF which was recognized clinically at Thi-kar governerate 1996.These sera include samples taken from the patient's mother and wife who were nursing him till death. They showed high levels of CCHF virus antibody and used as positive control serum in the study according to Gonzales et al 1990. [14]

Determination of anti-CCHF virus antibody: An ELISA technique was used for the detection of CCHF virus IgG antibody according to Shakerchi and Sever (1985) method.[15] Briefly, BPL-CCHF ELISA antigen donated by WHO to Iraqi medical authorities was coated to the polystyrene plate wells in an optimum antigen dilution of 1:100 which was selected together with 1:100 serum dilution through a chequer-board titration. After overnight incubation at 4C, wells were washed and loaded with 100/ml diluted sera and incubated at 37C for 1 hr. Plates were washed 4 times and goat-anti-human IgG, horse-radish peroxidase (Organon-teknica) conjugate (1:200) was added and incubated for 1 hr before washing. Substrate was added to the washed and dried plates for 30 minutes before stopping the reaction and read spectrophometericly using an ELISA reader at 492 nm. ELISA cut-off value was calculated by using positive and negative control sera and ELISA run to be valid at the P-N = 0.36 cut-off or more. Blood specimens with OD values less than the cut-off value considered negative. Sandwich ELISA was used for detection of anti-CCHF virus antibody in sheep and cattle sera according to Versteege (1995)[16] method using the respective anti-sheep and anti-cattle IgG horse-radish peroxidase conjugate (bioelisa, Spain). Then the procedure was completed as mentioned above.

RESULTS

Anti-CCHF IgG was detected in 6/8 (75%) of the suspected positive (P) sera obtained from the close contacts of 1996 CCHF fatal case. The highest OD value (P=1.80) was detected in serum obtained from the wife of died patient. These positive sera were pooled and used later as positive control with a mean OD value of 1.2. The negative (N) control represented by X OD value of PHL sera (X=0.22). The overall anti-CCHF IgG antibody prevalence among the resident of Basrah rural community was 4.3% (27/627). The sex prevalence for females was 1.6% (10/627) and for males 2.7% (17/627). Anti-CCHF IgG antibody was detected in 23/236 (9.7%) of zone A residents (Table-1). Seroprevalence of anti-CCHF IgG was 10.2% (14/136), 12% (6/50) and 6% (3/50) of individuals attending health centers at Hartha, Medaina and Qurna respectively. Individuals attending health centers at zone B(Zubair, Lehais and Safwan) were all seronegative, however, occupational serum samples of Zubair revealed anti-CCHF-IgG antibody prevalence of 3.6% (3/83). Safwan risk group samples were all seronegative. The overall IgG antibody prevalence of CCHF in zone B is shown in Table-1. Anti-CCHF IgG antibody was not detected in the localities of zone C. Similarly none of the risk group was seropositive except one at Shatt-Al-Arab area.

The results of ungrouped samples are presented in (Table-2), comparing sera from occupational animal contact with sera taken from PHL (non-contact). All of the 32 serum samples from persons subjected to AIDs screening program were seronegative for CCHF IgG antibody, while serum samples obtained from veterinarians, abattoir workers and farmers revealed anti-CCHF IgG prevalence of 17.39% (4/23).

Anti-CCHF virus IgG antibody prevalence in Basrah occupational risk group (animal contact) was detected in 12.19% (20/164) in serum

samples taken from individuals attending health centers (Table-3).

Animal serum samples tested by serum coating ELISA obtained from Hartha sheep revealed anti CCHF virus antibody in 20% (7/36) and in 37.5% (18/48) of cattles sera, while antibody prevalence in Lahais sheep was detected in 5.2%(2/38). These results are presented in (Table-4). None of the animals from other areas showed positive results for anti-CCHF virus antibody.

Tick's pools: *H. marginatum* was the most abundant and wide spread tick species in Hartha locality where pools identified indicate heavy animal infestation (over 10 ticks per pool). Of total pools collected from Hartha, 100% were *H. marginatum* and 100% of ticks identified were adults,male ticks were 68% and female were 32%. No nymph or larval stages were detected. Pools collected from Abu-Al-Khasib and Faiha were identified as *Boophilus annulatus* ticks. The incidence of the species *H. marginatum* in the overall collection from Basrah rural area were 85.7% (36/42) and *B. annulatus* were 14.3% (6/42). No ticks were observed in Lahais area (Table-5).

Table 1. Prevalence of anti-CCHF virus antibody among Basrah zones residents and its distribution according to sex.

Area	Locality	No. +/ No tersted (%)	Seropositive sex distribution	
			Female No. (%)	Male No. (%)
A	Hartha	14/136(10.2)	8(5.8)	6(4.4)
	Medaine	6/30 (12)	1(2.5)	5(10)
	Qurna	3/50	-	3(6)
	Total	23/236(9.7)	9(3.8)	14(5.9)
B	Zubair	3/83(3.6)	1(1.2)	2(2.4)
	Lehais	0/68	0	0
	Safwan	0/50	0	0
	Total	3/201(1.4)	1(0.49)	2(0.99)
C	Fao	0/50	0	0
	Abu-Alkhasib	0/50	0	0
	Shat-Alarab	1/50(2)	0	0
	Faiha	0/40	0	0
	Total	1/190(0.52)	0	1(0.52)
Total		27/627(4.3)	10(1.6)	17(2.7)
	Ungroubed	4/55(7.8)	0	4(7.8)

Table 2. Prevalence of CCHF virus IgG antibody in ungrouped samples and its distribution according to sex.

Type of sampling	No. tested	No. + (%)	Female N+ (%)	Male N+ (%)
PHL	32	0	0	0
Occupational	23	4(17.39)	0	4(17.4)
Total	55	4(7.27)	0	4(7.28)

PHL: Public health laboratory.

Table 3. Anti-CCHF virus antibody prevalence among occupational risk groups in Basrah and its distribution according to sex.

Sampling area	No. tested	No. + (%)	Female N+ (%)	Male N+ (%)
Zone A	58	12(20)	4(6.8)	8(13.8)
Zone B	43	3(6.98)	1(2.3)	2(4.66)
Zone C	40	1(2.5)	0	1(2.5)
Ungrouped samples	23	4(17.4)	0	4(17.4)
Total	164	20(12.19)	5(3.48)	15(9.14)

Table 4. Anti-CCHF virus IgG prevalence in sera of domestic animals in areas where CCHF cases were recognized (Hartha,Lehais).

Locality	Cattle's		Sheep's	
	No. tested	No.+ (%)	No. tested	No.+ (%)
Hartha	48	18(37.5)	36	7(20)
Lehais	0	0	38	2(5.2)
Total	48	18(37.5)	74	9(12.2)

Table 5. The incidence of the tick H. marginatum in certain localities of Basrah.

Locality	No. pools collected	H. marginat	Non H. marg.	Male (%)	Female (%)	Nymph or larva
Hartha	36	36(100)	0	68	32	0
Abu-Alkhasib	4	0	4	0	0	-
Faiha	2	0	2	0	0	0
Total	42	36(85.7)	6	68	32	0

DISCUSSION

In seroepidemiological surveys for arboviruses and viral haemorrhagic fevers, information is required for seropositivity in animals and humans who got the mild form of infections. These information with investigation of the vector distribution provide a profile for the virus circulation in its enzootic cycle.[2,7,17] In general the prevalence of anti-CCHF virus IgG antibody in Basrah rural community was 4.3% with a range fro 9.7% in zone A to 0.56% in zone C. These results were not far from that reported from south African rural community (1.2%-1.5%)[19,20], but less than that reported from Senegal (21%)[18] and Oman (30.3%). [8] The highest antibody prevalence (9.7%) obtained in this study indicates that human infection with CCHF virus occurs more frequently with less mortality in the region studied than has been found else where in Iraq. [11] However, in Senegal (21%) and Oman (30.3%) may be attributed to the difference in virus strain and the species of ticks available.[8,18] The prevalence of CCHF antibody in risk groups (animal contacts) were detected in all regions in Basrah rural community with higher prevalence toward localities of zone A. Hoogstraal (1981)[13] pointed out the importance of contact with domestic animals as a risk factor for human infection with CCHF virus in Eurasia. Moreover, many studies from Africa did not assure that, and concluded that physical contact with ticks represents major important mode of infection.[20,21] This may be due to difference in virus strain and its viremic level, and ability to infect vector through this route.[21,22] Animals get infections with CCHF virus only through tick bite. Serological surveys in animals, domestic or wild, represent a vital measure of enzootic focus, epidemic potential and reflect the activities of both the virus and the vector.[13,23] In this study the results of sandwich ELISA revealed antibody prevalence in cattle

sera(37.5%) and in sheep sera ranged from 20% in Hartha to 50% in Lahais .This was parallel to the result obtained from gathered ticks and the result obtained from human sera. These results are in consistence with 22% reported from Oman.[8] Fisher et al (1992)[19] found that antibody prevalence of 12.7% in young animals was as a result of physical contact with ticks. The ticks species *H. marginatum* was identified from all tick pools collected from Hartha (100%). The collection period was September 1996 till June 1997 which is an indicator of continuous ticks' activity through the months of winter and spring. However, following 1979 episode, Hoogstraal (1981) [13] stress the need for investigation of epizootology of CCHF virus and its threat to human health in Iraq, where several Hyalomma species thrive. So far there was no such study in this country that dealt with this subject as the present one. Many recent studies in Asia, Africa and Europe incriminated the genus Hyalomma as the principle vector for CCHF virus.[4,7,18,21] The data and information presented by this study had made a profile for the distribution of CCHF virus with strong evidence that its enzootic focus is potential one localized to the north region of Basrah and may form an epizoodemic network connecting other parts in southern Iraq. An investigation in the ecology of the enzootic focus provided an illustration of the connection between different epidemiological variables in the area. Wild vegetation provides food and shelter for different variety of wild mammals in the area. Previous studies by many investigators emphasized the role of wild animals in the transmission cycle of CCHF virus.[7,13,24,25] Economically and socially, domestic animal trading and transportation are common continuous practice for many inhabitants of Hartha locality, the later is considered as a station for housing animals from northern Basrah before sending them to slaughter house. Annual admission of the region by sheep shepherds from western Iraq (Al-Jazera) especially in winter seeking a free pasture for their animals permits sharing pasture area in the region. Moreover, the west-south of the region represents the free pasture zone that connects the north Saudi Arabia and wandering shephereds entering the area annually with their domestic animals. However, these animals (mostly camels) are potent host for numerous types of ticks species in their larval, nymph and adult stages.[28,29] The eastern part of the region extends with Iranian frontier and serologic evidence indicated that many of small mammals in the area were seropoisitive to CCHF virus.[28]

A cknowledgement:

Special thanks are due to Dr. Imad Abu-El-Eas of general public health laboratory, Baghdad for the donation of CCHF virus antigen used in this study.

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