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Aseroprevalance Survey of Anti-CCHFV Igg by ELISA in Sheep from Some Area in Northwest of Iran

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Abstract: Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic vector-born viral disease with a case fatality rate of 2-50% in human. CCHFV is classified within the *Nairovirus* genus in the Bunyaviridae family. The virus is endemic in over 30 countries, including Iran. Animals remove infect by virus without clinical symptoms. Among animals, sheep as the most important vertebrate hosts for (CCHF) virus is introduced. In present Study, the prevalence of (CCHF) virus in sheep in some regions of the North West of Iran is studied by serological ELISA method. Blood samples (131 female, 36 male samples) collected from four regions Uzan (20), Pesyan (24), Ibrahim Sami (18) and in suburb of Tabriz (105) [Mayan and Spyran] were used. Blood samples were collected via jugular vein, by Venoject without anticoagulant. After separation of serum, the sera samples have been stored at-20°C sent to Laboratory of Arboviruses and Viral Haemorrhagic Fevers (National ref. Lab) Pasteur Institute of Iran. Samples were classified to four grade Under 1 (30), 1-2 (16), 2-3 (28) and over 3 years (93) to evaluation the effect of age on seropositivity. Out of the 167 serum samples, 26 (15.56%) samples were positive for the presence of IgG [22 (17%) female and 4 (11%) male]. These results suggested that the virus is circulated in these regions. The age group over 3 year with 17 (18.3%) positive results was observed the highest positive serology at different ages. Ibrahim Sami [4 positive sera (22%)] the highest percentage of contamination region was reported. No significant relationship were found between regions and age with positive serology results were found (P>0.05). The entire situation recommended determining the disease in other areas sheep of East Azerbaijan, other livestock species in these regions and man will also be at risk.

Key words: CCHF · Iran · IgG · ELISA · Sheep

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) virus (genus *Naiorvirus*, family *Bunyaviridae*) is widely distributed in wild and domestic mammals such as human, birds and ticks throughout many regions of Africa, Europe and Asia [1, 2,3]. It was first observed in Crimea by Russian scientists in 1944 [1]. Crimean-Congo hemorrhagic fever (CCHF) may have been reported as early as 1110 AD, as per the description of a hemorrhagic syndrome associated with ticks in the *Thesaurus* of the Shah of *Khwarazm*, complied by *Dzurzhoni* [4]. The first evidence that CCHF circulated in Iran was investigated by Chumakov and his colleagues, at 1970, when 45% of a shipment of sheep sent from Tehran abattoir to Moscow tested positive for CCHF antigen [4]. Clinical CCHF was first recognized in Iran in 1999, with occurrence of the disease in several unrelated cases in different provinces, mainly Chaharmahal and Bakhtiari [9]. The diagnosis of CCHF was confirmed through detection of IgM, or IgG by ELISA and/or geonomic segment of virus by RT-PCR [1, 6]. The genome of CCHF virus could detect in the patients in saliva and urine [7]. The virus is a potential agent of bioterrorism [8]. The primary group of vectors responsible for human disease appears to be several species of the genus Hyalomma [1, 9]. During the recent years, outbreaks have been reported in South Africa, the Middle East and Iran [4]. Infection of the endothelium has an important role in CCHF pathogenesis. The endothelium can be targeted in two ways-indirectly by viral factors or

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virus-mediated host-derived soluble factors that cause endothelial activation and dysfunction and/or directly by virus infection and replication in endothelial cells. Endothelial damage contributes to haemostatic failure by stimulating platelet aggregation and degranulation, with consequent activation of the intrinsic coagulation cascade. Disseminated intravascular coagulation is noted as an early and prominent feature of the disease process [9, 10]. Some clinical finding and most common complaints in the patients is Myalgia, fever, lack of appetite, headache, nausea and/or vomiting, bleeding, diarrhea and cough, hepatomegaly, jaundice, rash, splenomegaly [11]. The aim of this study was to determine Anti-CCHF IgG in the serum of blood of sheep in some area of northwest in Iran.

MATERIALS AND METHODS

The samples were taken from 167 sheep in both sex in the 5 area of East Azerbaijan (Spyran, Myan, Ibrahim-Sami, Uzan and Pesyan). The blood was taken from Jugular vein by a Venoject (WeMed®, Hamburg, Germany) without anti coagulant. The serums were separated by a centrifuge with 3500 rpm in 10 minutes. Then the Sera samples were stored at-20°C were sent to Laboratory of Arboviruses and Viral Hemorrhagic Fevers (National ref. Lab) Pasteur Institute of Iran. The ELISA technique was used for detetion of IgG in the Serum samples. The ELISA plates were coated with the mouse hyper immune ascetic fluid (diluted at 1:1000 in phosphate-buffered saline (PBS 1x) and incubated overnight at 4°C. Following washing step, the native or the recombinant antigen diluted at 1:500 in PBS containing 0.5% Tween (PBST) and 3% skim milk (PBSTM), were 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-human or 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 incubation for each sample. Finally, hydrogen peroxide (H_2O_2) and 3, 3`, 5, 5` tetramethyl benzidine (TMB) was added and the plates were incubated for 15 min at room temperature. The enzymatic reaction was stopped by the addition of 4 N H_2SO_4. The plates were read by ELISA reader (Anthos 2020, Austria) at 450 nm [4, 8]. The analysis of results was done by SPSS (version 16) and $P<0.05$ was significantly for quantities data.

RESULTS

Out of 167 samples 26 (15.56%) was positive to CCHFV and had IgG in the serum. The positivity for IgG in the female and male were 22 (17%) and 4 (11%) respectively. It was no find significant correlation between sex and infection to the virus, so the sex was not

Table 1: Details of age group and the sex of the sheep

Age group and Sex	Under one year	1-2 years old	2-3 years old	Over 3 years old	Results	Sum
Female	2	11	24	72	Negative	109
Male	24	4	0	4		32
Female	0	1	4	17	Positive	22
Male	4	0	0	0		4
Sum	30	16	28	93		167

Table 2: Details of number and percentage (%) infection in each group

Group of age	Number of Samples	The Positivity	Percentage (%)
Under 1 year old	30	17	13.3%
Between 1-2 years old	16	4	6.25%
Between 2-3 years old	28	1	14.3%
Over 3 years old	93	4	18.3%

Table 3: The area of sampling from the sheep and positivity of samples or infection

The name of Area or village	The number of samples	The positivity	The percentage (%)
Spyran and Myan (suburb of Tabriz)	105	14	13.33%
Pesyan	24	4	16.66%
Ibrahyim-Sami	18	4	22%
Uzan	20	4	20%

a risk factor in this study (P>0.05). The samples were categorized in 4 groups (under 1, 1-2, 2-3 and over 3 years old) for study of the effect of age with the infection. The group of over 3 years old had the highest infection with 4 (18.3%) number and the lowest was in the group of 1-2 years old with 4 (6.25%) samples, but in the Chi-square test, it was no significant correlation (P<0.05). Analysis of the area with positivity shows that the highest infection was in Ibrahyim- Sami with 4 (22%) from 18 samples and the lowest was in the suburb of Tabriz (Spyran and Myan) with 14 (13.33%) from 105 samples. Furthermore in the Chi-square test was no significant correlation between area and the n umber of infection in sheep (P>0.05). Table 1-3 presented the details of results.

DISCUSSION

In this study 15.56% of blood samples were positive and IgG detected to the CCHFV. The first evidence that CCHF circulated in Iran was investigated by Chumakov and others (1970) when 45% of a shipment of sheep sent from Tehran abattoir to Moscow tested positive for CCHF antigen [4]. In other study that was done by Saidi [12], in North and West of Iran was positive to CCHF 62% and 28% respectively. That time they could not find any antibody in the serum of blood in the human. The result of our study was lower than the results of Saidi [12] who done the study by AGID [12]. Arata [13] were reported that 62% and 28% serum of sheep (average 54%) was positive for CCHF [20]. Chinikar [14] was reported that 31.26% of serum from domestic animals was positive for antibody of CCHF [5, 14]. Based of above reports, the present study shows the infection in the Northwest of Iran was lower (15.56%) nowadays. Some important factors might be influence of disease and infection rate are; the annually program against ectoparasite, population of sheep in the area and the industrial or traditional methods of animal husbandry. Geographical distribution of human cases of CCHF occurring in Iran and neighbors were reported by Chinikar S. and colleagues in 2010 [15]. The important points in mentioned report were that the Pakistan, Afghanistan, Iraq, Kazakhstan and United Arab Emirates had the highest infection cases of human [15]. In a study that was done in Egypt, out of 270 sheep examined, 17 (6.30%) were confirmed to have anti-CCHF IgG with highest titre recorded at 1:800. Also these authors found that the positive case in age over 2 years were significantly higher than those in age group less than 2 years [16]. In present study also the infection was increase with age. Izadi and his colleagues [17] reported the infection during 2004-2005 was positive (IgG) in the 107 (54.2%) of sheep blood samples that were taken that years. Mostafavi [18] were reported that the study between 9 years (1999-2008) on 4525 animals from 99 areas in Iran that collected from 26 provinces, 58/7% of sheep, 25% of cattle and 24.8% of goats was positive against for CCHF. Present study suggested the diagnosis of anti-CCHF IgG in blood would be done on the human population at risk of CCHF (such as veterinarian, slaughterhouse workers, farmers, hospital personnel, blood transfusion centers) and other animal such as cattle and goat and other sheep in other part of our area [3, 19].

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