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 Nairovirus, 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, compiled 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 genomic 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
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 ascetic (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 (H2O2) 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 H2SO4. 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>
<thead>
<tr>
<th>Group of age</th>
<th>Number of Samples</th>
<th>The Positivity</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 1 year</td>
<td>30</td>
<td>17</td>
<td>13.3%</td>
</tr>
<tr>
<td>Between 1-2 years old</td>
<td>16</td>
<td>4</td>
<td>6.25%</td>
</tr>
<tr>
<td>Between 2-3 years old</td>
<td>28</td>
<td>1</td>
<td>14.3%</td>
</tr>
<tr>
<td>Over 3 years old</td>
<td>93</td>
<td>4</td>
<td>18.3%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>The name of Area or village</th>
<th>The number of samples</th>
<th>The positivity</th>
<th>The percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spyran and Myan (suburb of Tabriz)</td>
<td>105</td>
<td>14</td>
<td>13.33%</td>
</tr>
<tr>
<td>Pesyan</td>
<td>24</td>
<td>4</td>
<td>16.66%</td>
</tr>
<tr>
<td>Ibrahim-Sami</td>
<td>18</td>
<td>4</td>
<td>22%</td>
</tr>
<tr>
<td>Uzan</td>
<td>20</td>
<td>4</td>
<td>20%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Age group and Sex</th>
<th>Under one year</th>
<th>1-2 years old</th>
<th>2-3 years old</th>
<th>Over 3 years old</th>
<th>Results</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>2</td>
<td>11</td>
<td>24</td>
<td>72</td>
<td>Negative</td>
<td>109</td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>17</td>
<td>Positive</td>
<td>22</td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>30</td>
<td>16</td>
<td>28</td>
<td>93</td>
<td>167</td>
<td></td>
</tr>
</tbody>
</table>

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 number 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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