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Seroprevalence of Crimean Congo Hemorrhagic Fever Virus in Livestock, Pakistan

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ABSTRACT

Crimean-Congo Haemorrhagic Fever Virus (CCHFV) is among the deadly human pathogens which cause a highly lethal haemorrhagic fever. CCHFV, a high-priority zoonotic pathogen is distributed widely and is transmitted in a vertical transmission cycle through these animals. Humans get infected by an infected tick bite, contact with viremic livestock blood, and through nosocomial route. Several CCHFV outbreaks have been reported for the past 2 decades in Pakistan and the virus has emerged in previously non-endemic regions as well. It is important to screen animals for CCHFV through an efficient diagnostic assay to prevent the viral zoonotic spill over to humans. **Objectives:** To screen the presence of CCHFV in sera collected from cattle, goat, and sheep in various regions in Punjab, Khyber Pakhtunkhwa (KP) and Sindh through a pre-established IgG ELISA assay. **Methods:** A recombinant nucleoprotein (rNP) of CCHFV was used to capture the anti CCFHV IgG antibodies in the animal sera. **Results:** Among 164 animals tested, 65% (103/164) showed the presence of IgG CCHFV antibodies. From the total 103 animals tested positive, 14.5 % (CI 0-28.2%) were cattle, 63.7% (CI 38.5-60.3%) were goats and 42% (CI 24.4-48.8%) were sheep. **Conclusions:** High seroprevalence of the CCHFV was expected from these areas as numerous cases of CCHFV have been reported previously. Since no commercial tests are available for the detection of CCHFV-specific antibodies in animals, this IgG ELISA test can be used to screen the animals in areas at risk such as those that have the presence of permissive ticks.

INTRODUCTION

Crimean The Crimean Congo Hemorrhagic fever virus belongs to the family *Nairoviridae* and genus *Orthonairovirus* and is one of the most prevalent tick-borne viruses [1]. CCHFV is the causative agent of severe haemorrhagic disease in humans with a high fatality rate of up to 30% [2]. Several tick genera can become infected with CCHFV however ticks of the genus *Hyalomma* are the principal vector [3]. Many vertebrate hosts like dogs, cattle, sheep, hares etc. get infected by the bite of infected ticks and the virus remains in their bloodstream for about one week after infection, allowing the tick-animal-tick cycle to continue when another tick bites. Humans acquire this infection from bite of an infected tick or by close contact of blood from an infected animal. Moreover, human to human transmission is also common in nosocomial setting. In animals, CCHFV infection shows mild or no clinical symptoms, but they do develop viremia that can last around 15 days [4] however in humans, the infection shows severe symptoms with high fatality rates if left untreated [5]. In humans, CCHF is manifested by fever, headache, vomiting, diarrhea, and muscular pain and bleeding diathesis with multiorgan dysfunction is observed in more severe cases [6]. Of the epidemic-prone diseases prioritized by the WHO R&D Blueprint, CCHF is the most widespread and is found in

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around 30 countries. CCHFV is distributed throughout the world and several outbreaks have been reported worldwide [7-8]. The incidence of this infection is consistent with the presence of the permissive ticks, Hyalomma, present in the geographical location [9]. The presence of *Hyalomma* ticks have been reported in many areas throughout Pakistan as well. A licenced vaccine or specific treatments are still not available for CCHFV immunization. Ribavirin is used as a general treatment, but it only helps alleviate the infection if it is given at an early stage hence it is important to diagnose the infection at an early stage. Pakistan is mainly an agricultural country and hence livestock forms an important part of the economy. Close contact of animals with humans is a major cause of spread of CCHFV infection. People in rural areas are particularly at risk because they are mostly employed with animal husbandry. Moreover, there is an influx of animals throughout the country during Eid ul Adha so the risk of infection is higher during that time. Furthermore, the recent political unrest in the country has also played a role in the spread of CCHFV as there has been an influx of refugees from Afghanistan which is a CCHFV endemic area [10]. Regular screening of ruminants and domestic animals help identify the geographical areas where CCHFV circulates and can also prevent the spread of infection to unsuspecting human [11]. The first CCHF case was reported in Pakistan in 1976 and since then many mini outbreaks have happened especially in Baluchistan [12]. In this study animal blood samples from various regions in Punjab (Islamabad, Rawalpindi), KPK (Kurram agency), Sindh (Tando Allahyar, Karachi, Larkana, Hyderabad) were tested using enzyme linked immunosorbent assay (ELISA) reported previously to have high sensitivity and specificity for the detection of CCHFV directed IgG antibodies [12]. A recombinant nucleoprotein of the CCHFV belonging to a Chinese strain 8402 sourced from Department of Veterinary Science, National Institute of Infectious Diseases, Japan was used to capture the IgG antibodies directed against CCFHV. This nucleoprotein is relatively conserved and can be used to detect many strains of the CCHFV. This assay is cost effective and can be replicated easily in labs with limited facilities such as those in rural areas. Assays available commercially are largely intended for use in human so this assay will help with serological screening of animals which is very important in the one-health approach.

METHODS

For this study, 164 animal serum samples from regions including Karachi, Islamabad, Rawalpindi, Tando Allah yar, Hyderabad, Larkana and Kurram Agency were screened for the presence of anti CCHFV IgG antibodies. The animal blood samples were collected in collaboration with Pakistan Agricultural Research Council (PARC). The samples were heat inactivated at 60 °C prior to testing to destroy any viral particles present [13]. The ELISA was carried out as previously described [12]. Briefly, the ELISA plates were coated with 100µl per well of the antigen (1: 400 dilution) and incubated overnight at 4 °C. The plates were washed with Tween20-Phosphate Buffered Saline solution (PBS-T) and inoculated for an hour using a blocking solution (PBS containing 0.5% Tween-20, 500 µg dextran sulfate/ml and 5% heat-inactivated horse serum). Following a third PBS-T wash, test samples that had been diluted four times from 1: 100 to 1: 6,400 were added to the plates. The samples were first incubated for an hour at 37 °C, followed by three PBS-T washings. Following that, the plates were infected for an hour at 37 °C with an anti-cow/goat antibody labelled with HRPD (1: 1000 dilution, Zymed). The optical density was measured at 405 nm after the plates had been cleaned and inoculated with ABTS (2,2 -azinobis 3-ethylbenzthiazolinesulfonic acid) solution for 30 minutes at room temperature in the dark. By deducting the OD of the matching coated well from that of the noncoated wells, the adjusted OD was computed. Control sera and the cutoff were used to determine the mean and standard deviation (SD).

RESULTS

A recombinant CCHFV nucleoprotein antigen was used to capture the CCHFV specific antibodies present in the serum. The cut off value was calculated by mean plus 3(SD) which was 0.213 at 1: 400 dilution. Out of total 164 animal serum samples tested, 103 (63%) were positive for presence of anti CCHFV IgG. From the total 103 animals tested positive, 14.5 % were cattle 63.7% were goats and 42% were sheep. Sero-prevalence of Crimean Congo Hemorrhagic Fever (CCHF) in serum samples of sheep, goat and cattle and its gender distribution collected from various regions and analysed using ELISA is shown in (Table 1).

Animal Species	Male	Female	Overall Sero-prevalence (%)	
	Positive	Positive	Total	95 % CI
Sheep	2/3 (66.7)	36/57 (63.2)	60 (36.6)	24.4-48.8
Goat	8/11 (72.7)	42/70 (60)	81 (49.4)	38.5-60.3
Cattle	1/1 (100)	14/22 (63.6)	23 (14)	0-28.2
Total	11/15 (10.7)	92/149 (89.3)	164 (100)	100

Table 1: Sero-prevalence of Crimean Congo Hemorrhagic Fever (CCHF) in serum samples of sheep, goat and cattle

The values in parenthesis are percentages. An overall sero-conversion of 62.8% (103/164) was observed in animals. The sero-conversion against CCHFV infection was highest in goat (49.4%), followed by sheep (36.6%) and cattle (14%) [Table1]. The eepidemiological and statistical characteristics between CCHFV positive and negative cases in ruminants is shown in [Table 2]. The sero-conversion against CCHFV infection was 89.3% in female,

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while it was 10.7% in male animals. The sero-conversion against CCHFV infection was highest female animals (90.9%). The CCHFV infection in animals of age groups ≤3, 4-6 and ≥7 years was 61.2%, 29.1% and 9.7%, respectively. The animal species, gender and age groups were not significantly associated with CCHFV infection. The location/area of sampling was found to be highly significantly associated with CCHFV infection ($p<0.05$). The highest seroconversions were observed Islamabad/Rawalpindi (57.3%) followed by Tando Allah Yar (17.5%), Kurram Agency (13.6%), Karachi (7.8%), Larkana (1.9%) and Hyderabad (1.9%) (Table 2).

Brief History	Total n=164 (100)			P- value
	Total n=164 (100)	Positive n=103 (62.8)	Negative n=61 (37.2)	
Gender				
Female	149 (90.9)	92 (89.3)	57 (93.4)	0.278
Male	15 (9.1)	11 (10.7)	4 (6.6)	
Animal Species				
Sheep	60 (36.6)	38 (36.9)	22 (36.1)	0.949
Goat	81 (49.4)	50 (48.5)	31 (50.8)	
Cattle	23 (14)	15 (14.6)	8 (13.1)	
Age groups (Years)				
≤3	101 (61.6)	63 (61.2)	38 (62.3)	0.776
4-6	49 (29.9)	30 (29.1)	19 (31.1)	
≥7	14 (8.5)	10 (9.7)	4 (6.6)	
Location (Area)				
Hyderabad	9 (5.5)	2 (1.9)	7 (11.5)	0.001*
Islamabad/ Rawalpindi	92 (56.1)	59 (57.3)	33 (54.1)	
Karachi	13 (7.9)	8 (7.8)	5 (8.2)	
Kurram Agency	20 (12.2)	14 (13.6)	6 (9.8)	
Larkana	10 (6.1)	2 (1.9)	8 (13.1)	
Tando Allah Yar	20 (12.2)	18 (17.5)	2 (3.3)	

Table 2: Epidemiological and statistical characteristics between CCHFV positive and negative cases in ruminant. *Pearson Chi-Square; values in parenthesis are percentages.

DISCUSSION

Detection of antibodies against CCHFV usually require the use of viral antigen or the viral particle itself to capture the antibody, this necessitates the use of biosafety level 4 labs with high containment levels which is not easily accessible especially in third world countries where cases are mostly reported. Use of a recombinant nucleoprotein that is noninfectious can be of great use to researchers and diagnostic labs for easy and safe handling and detection of CCHFV infection. A safe to handle recombinant nucleoprotein was used in this study for anti-CCHFV antibody capture. Moreover, the amino acid homologies are high among different strains in the region of the nucleoprotein used in this study, so this test can detect multiple strains of CCHFV. Our study shows a high proportion of seropositive animals from areas such as Islamabad/Rawalpindi and other areas in Sindh such as Tando Allah yar and Larkana which is in concordance with recent reports of CCHFV from these areas [14, 15]. The rural economy of Baluchistan and Khyber Pakhtunkhwa is based on livestock production, and the increased contact with animals may explain the higher antibody prevalence in humans from these areas. No significant difference was observed in the ratio of positive cases amongst different species of animals, most probably owing to the small sample size. The study can be carried out on a larger scale and with more animal species which will help identify areas more at risk of CCHFV prevalence. The rising geographic range of Hyalomma ticks is a concern, according to current studies. Additionally, migratory birds spread Hyalomma ticks into northerner regions of Europe, potentially exposing impressionable human populations to CCHFV. In the past five years, the virus has been brought into the UK on two separate occasions, with the first fatal case being confirmed in 2012. There isn't an approved vaccination for CCHF. In Pakistan most of the CCHFV cases are not diagnosed and if diagnosed, they are not reported [13, 16-18]. The farmers, shepherds and healthcare workers in Pakistan need to be educated about CCHFV transmission. There is an immediate need for a surveillance system, standard measures for early detection, preventive measures, and proper treatment plans. Future efforts for screening the animals using efficient diagnostic assays will be helpful to manage and curb this lethal zoonotic disease in humans. For accurate diagnosis of CCHFV, a simple IgG antibody detection assay is not enough. An IgM detection assay in addition to molecular detection of CCHFV antigen with qPCR will help accurately and efficiently diagnose the acute infection. Previous studies have shown that mononuclear phagocytes are one of the main targets of CCHFV [2, 19, 20]. Therefore, to increase the sensitivity of the ELISA assay whole blood samples may be used instead of serum, however more studies are needed to quantify the exact magnate of the CCHFV in Pakistan to help to begin right track for controlling the disease.

CONCLUSIONS

High seroprevalence of the CCHFV was expected from these areas as numerous cases of CCHFV have been reported previously. Since no commercial tests are available for the detection of CCHFV-specific antibodies in animals, this IgG ELISA test can be used to screen the animals in areas at risk such as those that have the presence of permissive ticks.

Conflicts of Interest

The authors declare no conflict of interest

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