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Full Length Article

Sero-Epidemiology of Crimean Congo Hemorrhagic Fever Virus (CCHFV) in Human and Livestock Population in District Mardan, Pakistan

Abdul Sajid[1*], Muhammad Ali[2], Hazir Rahman[2], Natasha Kashif[6], Hammad Ullah[1], Irshad Ahmad[3], Nighat Nawaz[4], Farhan Anwar Khan[5] and Fazal Rabbani[1]

[1]*College of Veterinary Science and Animal Husbandry, Abdul Wali Khan University Mardan, Khyber-Pakhtunkhwa, Pakistan*
[2]*Department of Microbiology, Abdul Wali Khan University Mardan, Khyber-Pakhtunkhwa, Pakistan*
[3]*Institute of Basic Medical Sciences, Khyber Medical University Peshawar*
[4]*Department of Chemistry, Islamia College University, Peshawar*
[5]*College of Veterinary Sciences, The University of Agriculture Peshawar*
[6]*Department of Microbiology, Abasyn University Peshawar*
[*]For correspondence: sajidvet137@awkum.edu.pk
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Abstract

Crimean Congo Hemorrhagic Fever (CCHF) is a viral zoonotic tick-borne disease caused by CCHF virus and extensively circulating in domestic and wild animals. The virus is transmitted to livestock population *via* bite of Hyloma ticks and to human through direct contact with blood of verimic animals or body fluid. The present study was performed to determine the seroprevalence of IgG antibodies to CCHF in human and livestock population and associated risk factors among both populations. A total of 25 union councils were selected in District Mardan in which 72 households/ Farms were visited. A total of 664 serum samples were collected from both populations (Livestock n = 543, Human n = 130). All farmers were extensively interviewed to identify major risk factors. A total of 447 serum samples were screened for the presence of IgG antibodies in both populations. A total 13% (n = 58) prevalence was recorded in livestock population. The highest ratio was detected in buffalo (19.3%) followed by Cow (15%), Sheep (15%) and goat 10.9%, respectively. While no positive sample was detected in human (n = 0). Moreover, among all tehsils in District Mardan, Tehsil Takht Bhai was highly exposed to CCHF having 15% prevalence. The lowest rate was detected in Tehsil Mardan (11.5%). It was observed that Hyloma ticks circulating in all households are on the top. All individuals were in close contact with livestock. The lack of population unawareness intensifies the risk of CCHF and its transmission. On the basis of above facts, it is concluded that there is a high ratio of CCHF in livestock population. If proper preventive measures were not adopted on time, it may cause a huge outbreak.

Keywords: Crimean Congo Hemorrhagic Fever Virus; Seroprevalence; Cross-sectional; Risk Factors; Livestock; Human

Introduction

Crimean Congo Hemorrhagic Fever Virus (CCHF) is a viral zoonotic disease mainly caused by Crimean Congo Hemorrhagic Fever Virus. The taxonomy shows the classification of viruses in order Bunyavirales, family Bunyaviradae, genus nairovirus (Whitehouse 2004). CCHFV generally belongs to the family Bunyaviradae of genus nairovirus that includes other genera *i.e*., Tospo virus, Hunta Virus, orthobunyavirus and Phlebo Virus. Bunyaviruses is different from genus nairovirus on based of their differentiation in L segmeng.one of their other serogroup named as Hazara Virus (HAZV) which was 1st isolated in Hazara region Pakistan (Donets *et al.* 1977; Wang *et al.* 2012). All of the 32 members of the Nairovirus genus are transmitted by argasid or ixodid ticks. The viral genome contains 3 segments of single-stranded, negative-sense RNA. Moreover, The CCHFV is spherical in shape having 80–100 nm of diameter, 8–10 nm of glycoprotein spikes presents on their surface and 5–7 nm thick lipid envelop. The genome of CCHFV is formed of negative ssRNA that have three segments. One is L-segment that encodes 450KDa-RNA dependent RNA polymerase (L-RdRp), second is M-segment which encodes 1700 amino acid precursors mainly used for the production of viral glycoprotein having mature GC (75KDa) and GN (37KDa) proteins and last is S segment which enclosed Nucleocapsid Proteins (Bergeron *et al.* 2015).

Furthermore, CCHF was first identified in a period of 12th century. The first CCHF outbreak was occurred in

1944, when various cases were found in soviet military personals (Hoogstraal 1979). They called the disease as Crimean Hemorrhagic Fever. After that, in 1969 the virus was isolated from patients in Congo showing similarity with Crimean Hemorrhagic Fever. Later on name was adopted as Crimean Congo Hemorrhagic Fever (Simpson *et al.* 1967).

The pathogenicity of CCHFV is not well understood. No clear pathogenesis is still described in CCHFV. It is asymptomatic in animal and birds but it shows their effect in human body when virus interacts with host is most responsible for the pathogenesis. The virus directly interacts or contributes with endothelial cell while indirectly contributing *via* immune cells. The over regulation of pro-inflammatory cytokines and soluble molecule that directly activate ECs which further goes to severity and may lead to cause hypotension, increased vascular permeability, vasodilation, shock, multiple organs failure and then lead to death (Akinci *et al.* 2013).

Several ixodid ticks, especially those of the genus Hylomma are both reservoir and vector for the CCHF virus and play important role in the ecology and epidemiology of CCHF (Khurshid *et al.* 2015). Hence, dealing with this type of ticks can increase risk of infection. Infection generally occurs and spreads by farming activities and interrupting ticks' population. Migration of ticks-invaded animals or birds may lead to population increase. Two species of ticks mainly cause CCHF. Species include soft ticks (family Argasidae) and hard ticks (family Ixodidae). Also virus has been isolated from various other tick's genera, which are Ixodes, *Rhipicephalus* spp. and Dermacentor.

The virus is transmitted to human population by infected ticks, direct contact with fresh meat or blood of variemic animals (domestic) or direct contact with blood or secreted fluid of an infected human. Migration of infected animals can increase the risk of infection in other areas (Altizer *et al.* 2011; McKay and Hoye 2016). Nosocomial transmission of CCHF among healthcare workers through the CCHFV antigen from infected patients by plasma, tissue or blood is possible. Different methods are used for diagnose of CCHF include Enzyme Linked Immunosorbent Assay (ELISA) for serological identification and Reverse Transcription Polymerase Chain Reaction (RT-PCR) for molecular identification (Whitehouse 2004).

Material and Methods

Study area

The study was conducted in district Mardan, which is the 2nd largest city of Khyber Pakhtunkhwa. There are four Tehsils and 72 union councils with various villages. The study was based on cross sectional survey to determine the seroprevalence and factors associated with risk of CCHF in Human and livestock population in district Mardan.

A total of 25 union councils from Mardan were selected for sample collection. In these union councils 72 Households/Farms were visited where various population of human was in contact with livestock's population.

Sample collection and transportation

Overall, 664 blood samples (Livestock n = 534, Human n = 130) were collected. Moreover, from each of individual, 4–5 mL of blood was collected in a gel containing tube. All samples were transported under refrigerated condition to the Veterinary Pathology Laboratory of CVS & AH. Blood samples were then incubated at room temperature for 6–8 h. After incubation, all samples were centrifuged on 5000 rpm for 5 min for pure serum collection. Serum of each sample was transferred to Eppendorf tube (1.5–2 mL) and stored at -20°C till further use.

Data survey

Concerned persons/ farmers of each household were extensively interviewed. The interview based on questionnaire/survey form was consisting of 46 questions. The survey form covered demographic characteristics; occupation of livestock handler, household's data, history of animals, tick interaction and knowledge about CCHF. One or two concerned humans of each household were extensively interviewed and factors favoring to increase the zoonotic infections were documented.

Serology

The ID Screen CCHF Double Antigen Multi-species ELISA kit (ID-Vet France) was used for detection of antibodies against Crimean Congo Hemorrhagic fever virus in collected serum samples. About 447 randomly selected serum samples (Livestock = 381 & Human = 66) were tested on indirect ELISA kit to identify the IgG seropositive cases of CCHFV.

Statistical analysis

Significance different was assessed by using chai square test.

Results

In the present study a total 58 (13%) animal serum samples were found positive for CCHFV antibodies by a single step approach using indirect ELISA technique.

The positive samples detected in cow, Buffalo, goat and sheep were 23 (14%), 24 (19.3%), 8 (10.9%) and 3 (15%), respectively (Table 2; Fig. 1).

Tehsil wise seropositivity

Serological evidence was recorded in 24 samples from tehsil mardan (11.5%), 15 Samples from Tehsil Rustum (14.7%),

Table 1: Specie Wise Seropositivity in Livestock Population in District Mardan

S. No.	Specie	Total tested Sample	No. of Positive Sample	Prevalence (%)	*P* value
1.	Cows	164	23	14	0.414
2.	Buffalo	124	24	19.3	
3.	Goat	73	8	10.9	
4.	Sheep	20	3	15	

Table 2: Tehsil wise seroprevalence of CCHFV in district Mardan

S. No	Tehsil	Total tested Sample	No. of Positive Sample	Prevalence(%)	*P* value
1.	Mardan	208	24	11.5	0.738
2.	Rustum	102	15	14.7	
3.	Takht bhai	80	12	15	
4.	katlang	57	7	12.2	

Table 3: Species and Area wise detail of data

Tehsil	Cow		Buffalo		Sheep		Goat		Total sample	
	+ive	-ive	+ive	-ive	+ive	-ive	+ive	-ive	+ive	-ive
Mardan	6	66	13	63	3	35	2	48	24	185
Rustum	8	52	6	37	0	0	1	7	15	87
Takht bhai	6	57	5	27	0	0	1	6	12	68
katlang	3	42	0	28	0	0	4	13	7	50

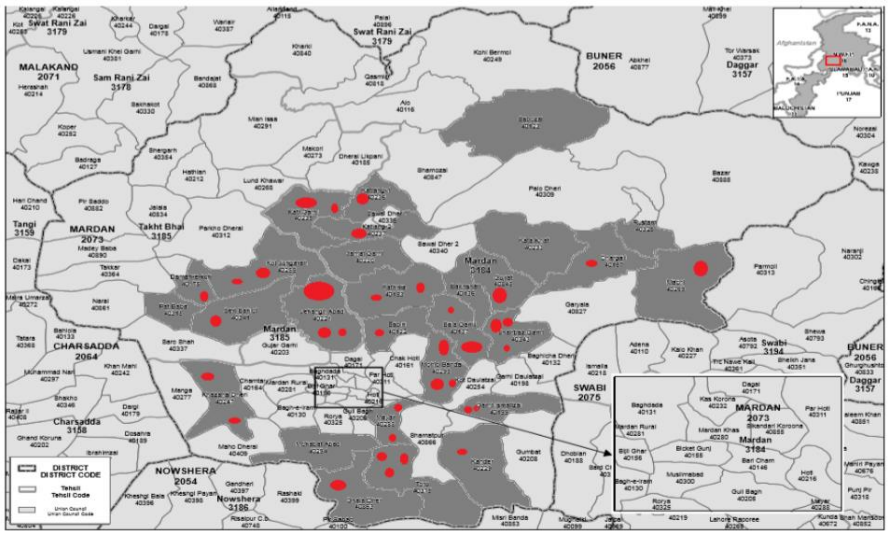

Fig. 1: Map of District Mardan Showing CCHF Positivity

12 samples from Tehsil Takhtbhai (15%) and 7 Samples from Tehsil Katlang (12.2%). The area and species wise highest seroprevalence were documented in buffalos from tehsil Mardan. While in cows the highest ratio was detected in tehsil Rustum followed by tehsil Takht Bhai. By comparing the ratio of positive cases in large and small ruminants it was documented that the lowest positive ratio of CCHF was found in small ruminants in all districts (Tables 1–3).

Associated risk factors

From all the 25 villages/union council of district Mardan, 72 households/ farms were visited with local veterinarians to identify the major risk factors associated with animals and humans (Table 4 and 5).

By concluding the above analyzed data, it was found that people living in district mardan are at high risk to CCHF infection. Major associated risk factors were the presence of ticks on animal (73.6%), ticks manual removal from animal body (52.7%), keeping animals inside the living room (8%) and house (68%) where the farmer resides. But the most important and major risk factor identified in the study was the unawareness, lack of knowledge about CCHF (79.1%) and its transmission (97.2%). Other risk factors identified were careless slaughtering, animals handling without PPEs, carless attitude towards hands washing after contacting animals and use of unpasteurized milk. The knowledge about zoonotic and nosocomial transmission was 2.7%. This factor can contribute much to cause alarming outbreaks.

Table 4: Demographic characteristics of participants

Variables	Categories		n (%)
Gender	Male		72 (100)
	Female		0
Age group	(12-25)		9 (12.5)
	(25-35)		37 (51.3)
	(35-45)		15 (20.8%)
	(45-55)		11 (15.2%)
Married	Yes		87.5(87.5%)
	No		9 (12.5%)
Range of Family	(1-5)		18
Member in	(5-10)		41
Households	(10-15)		12
Range of Animals	Cow	1-5)	22
Living in		(5-10)	43
Households		(10+)	7
	Buffalo	1-5)	43
		(5-10)	16
		(10+)	13
	Goat	1-5)	15
		(5-10)	9
		(10+)	5
	Sheep	1-5)	1
		(5-10)	0
		(10+)	2
Occupation	Farmer		55 (76.3%)
	Butchers		7 (9.7%)
	Veterinarians		6 (8.3%)
	others		4 (5.5%)
Activities of individuals	Milk Cows/Buffalo		45 (62.5%)
	Milk sheep or goat		26 (36.1%)
	Prepare Milk Product		76 (52.7)
	Slaughtering animals and prepare meat		17 (23.6%)
	Helps at births time		66 (91.6%)
	Remove ticks from animals		63 (87.5%)
	Treat animals for ticks using insecticides		48 (66.6%)

Table 5: Knowledge and attitude of participants towards risk of CCHF

Variables	Categories	No (%)
Do your animals have Ticks?	Yes	53(73.6%)
	No	19(26.4%)
Do you Milk cattle's?	Yes	55(76.3%)
	No	17(23.6%)
How do you use milk?	Always boil before using	63(87.5%)
	Use without boiling	5(6.9%)
	Sell or Trade	48(66.6%)
	Do not use milk	4(5.5%)
How do you Treat your animals against ticks?	Dipping	11(15.2%)
	Spraying	23(31.9%)
	Manual	38(52.7%)
Do you Take any special precaution while handling animals	Using gloves	3(4.1%)
	Wash hands with soap and water	69(95.8%)
Do you keep animals Outside of your Home/Farm?	Yes	23(31.9%)
	No	49(68.0%)
Do you keep the animals in the room where you sleep?	Yes	8(11.11%)
	No	64(88.88%)
Have you ever feel any symptoms after tick bitten?	Yes	2(2.7%)
	Don't know	53(73.6%)
	No	17(23.6%)
Have you heard about Congo fever before?	Yes	15(20.8%)
	No	57(79.1%)
Do you think CCHF could be transmitted from one another?	Yes	2(2.7%)
	No	70(97.2%)
During the past month did any person in your households have CCHF?	Yes	0
	No	72(100%)

Major vulnerable groups include Farmers, butchers, veterinarians, veterinary assistants, health care workers and all those people living in endemic areas.

Discussion

Serological identification of CCHFV in ruminants allows to identify the affected areas. Prevalence of IgG antibodies is the main indicator of CCHFV circulation. Detection of IgG antibodies in domestic large and small ruminants indicates that livestock is a potential source of infection for human. In Pakistan, the CCHF is endemic and highest number of cases reporting after Turkey, Russia and Iran. With this non serious approach towards CCHF, it is expected that in near future the virus will infect many more and future losses will likely be much higher than present.

Keeping in view, the public health importance in connection with CCHF, this study was designed to identify the seroprevalence and risk factors of CCHFV in human and livestock populations. In the present study an overall prevalence of 13% (n = 58) in livestock population were recorded by using a single step approach of ELISA. It was a blessing that in such situation there was no single positive case in human population during the study time. Many studies have been reported from various regions of Pakistan and throughout the world that CCHFV is frequently present in cattle. It was observed in recent studies that all over Pakistan, the prevalence recorded is 36.2%. Moreover, Occupational based prevalence reports 3.2% in livestock farmer and 0.2% in general population (Zohaib *et al.* 2020).

By concluding the findings of the present study, it was noted that CCHF is somehow equally prevalent in all the species of livestock. Serologically positive indication was observed highest in buffalo (19.3%), followed by cow (14%), sheep (15%) and goat (10.9%). Livestock population is asymptomatic but can act as carrier of CCHFV to human population. Recently published data investigate a highest (56.7%) prevalence of CCHFV in camel in all provinces of Pakistan followed by cows (44.3%) and buffalo (29.6%). While in case of small ruminants, sheep indicate highest (32.6%) presence of IgG antibodies, while the lowest prevalence was recorded in goat (18.9%) (Zohaib *et al.* 2020). These studies support the conclusion of the present study that the CCHFV is prevailing in the country and human population being in direct contact could get the infection any time.

In district Mardan the highest prevalence (15%) was found in Rustum and Takhtbhai, while lowest ratio was recorded in Tehsil katlang. Area wise the highest prevalence was recorded in union councils of Tehsil Mardan and Takhtbhai. UC Mayar and UC Jahangir abad detected 30.7% of CCHFV positivity ratio. It means that CCHFV is circulating in all areas with alarming and high risk of CCHFV transmission. It is supported by a previous provincial level study of Pakistan That Baluchistan and

Khyber Pakhtunkhwa provinces have highest prevalence with 59.3 and 52.4% of CCHFV in livestock population. Punjab and Sindh provinces have reported 24.7 and 16.2% positivity, respectively (Zohaib *et al.* 2020). Another study conducted on ticks collected from livestock population excluding buffalos in Baluchistan, Pakistan by Kasi *et al.* (2020) reported that ticks with CCHFV were only recovered from sheep. The ticks infested all the livestock populations including cattle (14%) goat (28%) and sheep (58%).

The area wise prevalence in livestock population indicates that CCHFV is circulating in whole district. Previous data of CCHF cases in human indicates that these animals have a potential of CCHFV transmission to human. If the required preventive measures are not followed by farmers and general public to restrain the spread of CCHFV, we may witness a very disastrous outbreak and lambast human population with cataclysmic effects.

Conclusion

There was a high ratio of CCHF in livestock population, although these cases are present sporadically. But if proper preventive measures and awareness campaign have not been adopted on time, it may cause severe infections cause huge losses to the precious human population. The people should be aware of this fatal disease mainly of its transmission, clinical characteristics, public health importance, preventive protocols and case handling. The present study was being conducted on serological grounds therefore; it is recommended that a study using molecular techniques must be conducted in order to have a confirmed and actual rate of CCHFV prevalence.

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Author Contributions

Abdul Sajid and Muhammad Ali planned and performed the experiments, Hazir Rahman, Natasha Kashif, Hammad Ullah, Irshad Ahmad and Nighat Nawaz interpreted the results and made illustrations, Farhan Anwar Khan helped in few experiments and paper write-up while Fazal Rabbani helped in sample collection.

Conflicts of Interest

The manuscript has not been published in whole or in part nor it is being considered for publication elsewhere. The authors declare no competing interests exist.

Data Availability

The data that support the findings of this study will openly be available to all research community without any formal restrictions.

Ethics Approval

Serum samples were collected according to fundamental ethical principles for diagnostic purposes in context of national surveillance studies.

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