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CRIMEAN-CONGO HEMORRHAGIC FEVER OUTBREAK IN CHAKWAL, PAKISTAN

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*Crimean-Congo hemorrhagic fever (CCHF) is the most fatal viral disease with extensive geographical distribution. In Pakistan it is being reported with sporadic outbreaks in cattle rearing areas. The authors in this study presented a clinical case of CCHF in one of cattle rearing district Chakwal of Punjab Province. The serums and plasma samples from suspected patients along with Hyalomma ticks from reported area were collected and diagnosed for CCHF antigen and IgG antibodies by two step sandwich enzyme-linked immunosorbent assay (ELISA) using Vector BEST Company, Crimean-CHF-antigen kit, Novosibirsk, Russia. Crimean-Congo hemorrhagic fever (CCHF) was suspected in three patients among which two patients died. The only survived patient was diagnosed for CCHF by detecting IgG. Out of 62 Hyalomma ticks collected from livestock of endemic area, 9.67% (6/62) were positive for the CCHF virus. This confirms the emergence of CCHF virus in new areas and the potential risk of its wide spread in different areas of Punjab, Pakistan. **Keywords:** CCHF, tick, livestock, CCHF deaths, diagnostics, clinical symptoms.*

Геморрагическая лихорадка Крым-Конго (ССНФ) является наиболее смертельным вирусным заболеванием с обширным географическим распространением. В Пакистане сообщается о спорадических вспышках в районах, где интенсивно выращивается крупный рогатый скот. Авторы данного исследования представили клинический случай ССНФ в одном из районов, специализирующемся на разведении крупного рогатого скота - район Чаквал провинции Пенджаб. Сыворотки и образцы плазмы у подозреваемых пациентов вместе с клещами Hyalomma из сообщаемой области были собраны и диагностированы на антиген ССНФ и IgG-антител с помощью двухступенчатого сэндвич-фермент-связанного иммуносорбентного анализа (ELISA) с использованием Vector BEST Company, набора крымских-CHF-антигенов, Новосибирск, Россия. Геморрагическая лихорадка Крым-Конго (ССНФ) подозревалась у трех пациентов, из которых умерли два пациента. Единственный выживший пациент был диагностирован на ССНФ по обнаружению IgG. Из 62 клещей Hyalomma, обнаруженных на крупном рогатом скоте эндемического района,

9,67% (6/62) дали положительный результат на наличие вируса ССНФ. Это подтверждает появление вируса ССНФ в новых областях и указывает на потенциальный риск его широкого распространения в разных районах Пенджаба, Пакистан. **Ключевые слова:** гемморрагическая лихорадка Крымско-Конго, клещ, домашний скот, смертельные случаи, диагностика, клинические симптомы.

Introduction. Crimean-Congo hemorrhagic fever (CCHF) is endemic in Pakistan with sporadic outbreaks [1]. In Pakistan, it was initially reported in 1976 [2]. CCHF was first reported in Russian soldiers and peasants who were exposed to ticks in Crimea in 1944 [3]. CCHF is a zoonotic disease maintained in nature by cycles between reservoirs (ticks) and (largely sub-clinical infection in) mammals. The main vector of CCHF is the hard tick (Ixodidae) of Genus *Hyalomma* in which vertical and horizontal transmission of viruses also occurs within the tick population. *Hyalomma* species mainly present in small and large ruminates [4]. In Pakistan, more than 50% of tick infestation has been reported in livestock [5, 6]. In livestock, highest prevalence of *Hyalomma* species followed by *Boophilus*, *Rhipicephalus* and *Haemaphysalis* have been reported in various districts of Punjab, Pakistan i.e. Kasur, Khushab, Lahore, Rawalpindi, Sargodha and Toba tek Singh [7, 8, 9, 10]. Severe disease only occurs in humans, who usually acquire the virus through a tick bite or through close contact with infectious secretions of animals. The viral incubation period of 2–9 days is generally followed by fever and progressive lassitude and changes in behavior. The onset is usually abrupt and by day 4 the patients usually exhibit somnolence, depression, chemosis and conjunctival injection [11]. A petechial rash appears usually after the 6th day of infection followed by ecchymosis, purpura and large bruises especially in upper arms, axillae and antecubital fossa. Internal bleeding including intracranial and intraperitoneal bleeding may occur and patient may undergo deep sleep and coma eventually. The patient remains highly infective during this period. During the early course of illness any of the following clinical laboratory values are highly predictive of a fatal outcome: leucocyte count $\geq 10 \times 10^9/L$; platelet count $\leq 20 \times 10^9/L$; AST ≥ 200 U/L; ALT ≥ 150 U/L; APTT ≥ 60 seconds; and fibrinogen ≤ 110 mg/dL [12].

Materials and methods of the research. The District Chakwal (32.8322° N, 72.6151° E) is located 90 km South-East of the federal capital, Islamabad in the province of Punjab, Pakistan. During the period March to July, 2016, three cases were reported for CCHF. The first two cases were reported from the village Dhok Shah Gul Hassan Tehsil Lawa, and third case was reported from village Kharra Tehsil Chakwal, District Chakwal. It is pertinent to mention that both areas belong to Pothohar plateau and a cattle rearing is major profession in these areas. All three patients were from rural area and having close contact with livestock. Out of these three, two patients expired while one survived. Tick Collection, Identification and Detection for CCHF virus from endemic region : During physical examination of animal ticks were collected from the infested animals in the vicinity of reported cases. The ticks were collected in a separate labeled vial from each individual. Then these ticks were identified according to their morphological features i.e. anal groove, mouthparts, basis capituli, eyes, scutum, festoons etc. using standard morphological key [13]. The blood fed adult female ticks were stored at -80°C for antigen ELISA. Each vial containing the same tick from a single animal was tested as a pool. Hence a total of 62 pools were stored for testing. Out of 62 ticks, 9.67% (6/62) were positive for the CCHF virus antigen by two step sandwich enzyme-linked immunosorbent assay (ELISA) using commercially available kit (Vector BEST Company, Crimean-CCHF-antigen kit, Novosibirsk, Russia).

Results of the research. Case-1: A 45 years old female patient with a history of fever, body ache and GIT disturbances since 4 days and bleeding from gums since 2 days admitted to holy family hospital Rawalpindi during March 2016. She was referred to the hospital from private clinic in the Union Council Kot Qazi, Tehsil Lawa, District Chakwal. There she had been managed supportively for 3 days and a complete blood count (CBC) test was ordered. It shown decrease in platelet count with other counts in normal range. She belonged to a rural area and had the history of exposure with livestock on daily basis. However she could not recall a history of tick bite. She also not reported previously any history of bleeding diathesis. Upon admission her blood picture revealed further decrease in platelet count. Dengue serology for acute infection was negative. A clinical picture typical of hemorrhagic fever, a positive history of contact with domestic animals, and awareness of an ongoing CCHF epidemic prompted suspicion for CCHF. Her blood sample was sent to National Institute of Health (NIH) Islamabad on next day of admission and found positive for CCHF virus by using. While her stay in hospital she did not get stable and expired on 3rd day of admission. After her death on very next day the NIH report confirmed her for CCHF. Case-2: On very next of her death, her husband who is about 55 years also developed symptoms of fever, vomiting and GIT disturbances. He visited local private health care centre. On 3rd day of first appearance of symptoms he reported moderate hemoptysis. Platelet count was found decreased on CBC. He was also immediately shifted to holy family hospital Rawalpindi, the nearest tertiary care hospital in the area. During his stay at hospital he was kept in isolation unit. A strict nursing barrier protocols were followed, involving use of gloves, gowns and N-95 respirators at each patient encounter. The patient was discharged on the 14th day of admission with approximately 17 days of illness. Case-3: A 13.5 year old boy from Village Kharra, Tehsil Chakwal, and District Chakwal-with a history of high grade fever visited private health clinic. He was given supportive treatment for fever and a complete blood count (CBC) was ordered. Decrease in platelet count was observed. Next day he was admitted to Fauji Foundation Hospital Rawalpindi and was treated for fever with thrombocytopenia. On third day of illness, patient was unconscious and shifted to ICU and ventilator support was initiated. On 4th day, he was in state of coma. On suspicion of CCHF his blood sample was sent to NIH for the confirmation of CCHF virus. But unfortunately, patient died very next day of dispatching sample. However, sample was confirmed positive for CCHF after the death of patient.

This case also belonged to rural area and was exposed to livestock on daily basis. No history of tick bite was recorded but ticks collected from the animals (buck and cattle calf) at his home were positive for CCHF virus antigen.

Conclusion. Pakistan is endemic for CCHF with the report of sporadic cases. Deaths have been reported to occur on days 5–14 of CCHF fever [14]. The likely source of the CCHF identified in the village of kot qazi, Chakwal District was virus-infected Hyalomma ticks and livestock at the residence of the primary case. The CCHF patients were in close contact to those animals having ticks positive for CCHF virus.

Ticks are accepted as main transmission vectors for the virus to humans and susceptible animal species which act as amplifying host. Our results also indicate that ticks are the potential risk factor for CCHF infection. A total of 9.67% (62/6) ticks were positive for CCHF virus antigen. Our results indicate that human population in the surrounding of cases is at greater risk of CCHF and there is need to control the ticks in livestock population. Sero-surveillance of CCHF Virus in human and livestock population should be conducted to suggest control measure for the possible outbreak in future.

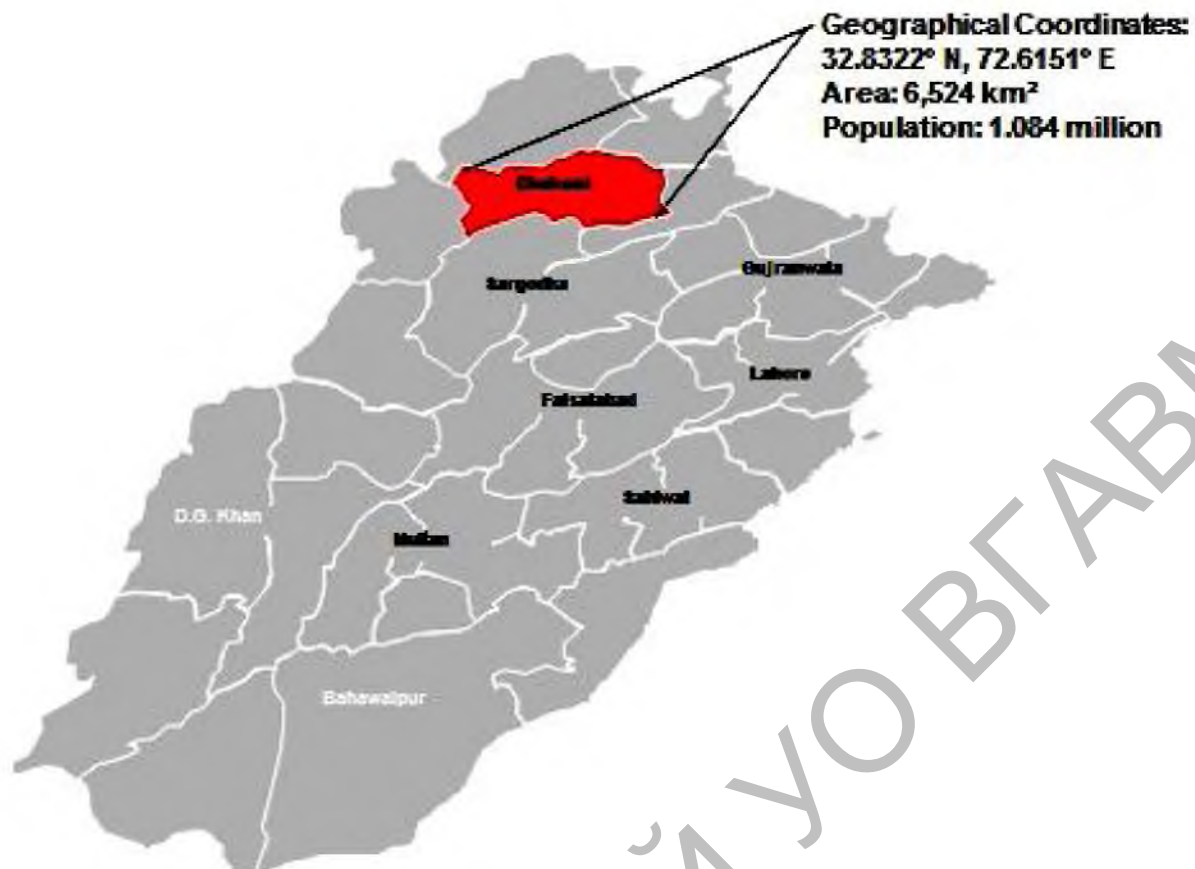


Figure 1 - Geographical coordinates of CCHF in Pakistan

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