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LETTER

Sero-Epidemiological Survey of Crimean-Congo Hemorrhagic Fever among the Human Population of the Punjab Province in Pakistan

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Dear Editor,

Crimean-Congo hemorrhagic fever (CCHF), caused by the CCHF virus (CCHFV), is a tick-borne zoonotic infection characterized by myalgia, high-grade fever (> 38 °C), headache, nausea, bleeding from the body cavities, and in 10%–50% of cases, results in death (Swanepoel *et al.* 1989). As CCHFV belongs to the *Nairoviridae* family, the virus can be transmitted to humans through the bite of infected ticks or by contact with the tissues or blood of infected animals (Bente *et al.* 2013).

Since the first case of CCHF in Pakistan in 1970, the number of clinical cases has increased annually (Yousaf *et al.* 2018). Like other resource-limited countries worldwide, most diagnoses of this disease in Pakistan are limited to clinical signs and symptoms (Haider *et al.* 2016; Hasan *et al.* 2013). Consequently, despite the endemicity of the disease in various regions of Pakistan, the majority of cases remain either undiagnosed and/or misdiagnosed, due to lack of diagnostic facilities. In order to highlight the presence of subclinical forms of the disease or underestimation of actual disease status and associated risk factors, we conducted a sero-epidemiological study involving patients originating from areas with and without a history of the disease.

We reported the presence of anti-CCHFV IgG from 1052 collected blood serum samples in selected districts of the Punjab Province of Pakistan (see Supplementary Materials and Methods for data collection and immunological assay details) over a period of 8 months (from October 2016 to May 2017) and found an association between seroprevalence and different categorical variables. We found a lower CCHFV seropositivity (2.09%, 95% CI 1.23–2.96), which is not surprising, as previous studies have found that CCHFV seropositivity varies even in endemic places. For instance, using sera of individuals that had a subclinical form of infection or were clinically healthy, the percentage of patients which were seropositive was found to be 0.5% in India (Mourya *et al.* 2019), 3.7% in Bulgaria (Christova *et al.* 2013), 4.9% in Greece (Sidira *et al.* 2012), 9.3% in Kosovo (Fajs *et al.* 2014) and 10%–19.6% in Turkey (Ertugrul *et al.* 2012; Koksal *et al.* 2014). Such variations in the study outcomes may be attributed to the sampling and subsequent analysis strategies employed previously. For instance, some of these studies included areas with a frequent occurrence of the disease reported in the past, while other studies involved a comparison between areas with a frequent occurrence to areas with no prevalence. For example, studies from Turkey were exclusive to patients originating from disease-endemic areas, whereas, similar to our study, both endemic and non-endemic areas were explored in the study from Kosovo.

Herein, we report a higher seroprevalence in regions having a history of exposure (n = 20/741, 2.70%, 95% CI 1.58–3.97), that was four times higher (OR = 4.29, 95% CI 0.99–18.45, $P = 0.03$) than that found in regions without any history of CCHF (n = 2/311, 0.64%, 95% CI 0.25–1.54) (Table 1). Among the districts with a history of CCHF, the Chakwal district had the highest prevalence (7.45%, 95% CI 2.14–12.75), followed by the Mianwali (3.48%, 95% CI 0.39–7.37), Rawalpindi (3.09%, 95% CI

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Table 1 Seroprevalence and associated risk factors for CCHFV.

Risk factors	Positive	Negative	Seropositivity (%)	*P* value	Odds ratio	95% CI
Age group						
18–40	8	568	1.39			
41–55	14	462	2.94	0.08	2.15	0.89–5.17
Gender						
Male	16	775	2.02	0.80	0.87	0.33–2.26
Female	6	255	2.29			
Occupation						
Farmer	15	453	3.20	0.02*	2.72	1.10–6.75
Other	7	577	1.20			
Habitat						
Rural	20	642	3.02	0.01*	6.04	1.40–26.01
Urban	2	388	0.51			
Exposure history						
Areas with history of CCHF	20	721	2.70	0.03*	4.29	0.99–18.45
Areas with no history of CCHF	2	309	0.64			

*Indicates significant values.

0.35–6.54), Lahore (2.63%, 95% CI 0.09–5.18), and Dera Ghazi Khan districts (1.98%, 95% CI 0.74–4.70), whereas, the lowest prevalence was found to be in the Attock district (1.01%, 95% CI 0.94–2.92) and no seropositivity was found in the Rajanpur district (Fig. 1). Similarly, in areas with no previous exposure, the seroprevalence was present in the Jhelum district (1.90%, 95% CI 0.71–4.52), while in the Bahawalpur and Rahim Yar Khan districts, no CCHFV seroprevalence was identified, as shown in Fig. 1. We found 7 of 10 districts with seropositive individuals, 2 districts without, and 1 district with a history of CCHF, indicating that sub-clinical forms of infection are also present in Pakistan and should, therefore, be included when conducting a mass-scale surveillance study in the future. However, seropositivity to CCHFV was more prevalent in areas with a history of CCHF.

In order to assess a relationship between risk factors and seropositivity to CCHFV in humans, different categorical variables, including age, gender, geographic characteristics (endemic/non-endemic), and occupational history, were analyzed (Table 1). Our results showed that among seropositive individuals, females were more common (n = 6, 2.29%, 95% CI 0.56–4.77) than males (n = 16, 2.02%, 95% CI 1.06–3.07) but we found a lack of gender predisposition against CCHFV seroprevalence ($P > 0.05$). This is contradictory to previous observations from India (Mourya *et al.* 2019) which may be due to the differences in the environment of the study participants in each country. For instance, both males and females included in our study had equal exposure to agriculture and livestock, however, females were involved mainly in livestock rearing and management. Similar observations have been reported by another study, where the percentage of seropositive patients was found to be higher in females who had direct contact with animals and, therefore, were exposed to tick-bites (Lwande *et al.* 2012). On the other hand, in the referenced Indian study, males were more exposed to livestock and agricultural activities than females.

The seroprevalence was 6 and 2.7 times higher in individuals living in rural areas (OR = 6.04, 95% CI 1.40–26.01, $P = 0.013$) and livestock farmers (OR = 2.72, 95% CI 1.10–6.57, $P = 0.029$), respectively. The percentage of seropositivity was higher in individuals involved in agricultural and livestock-related activities (3.20%, 95% CI 1.77–4.46) than others (1.20%, 95% CI 0.23–1.41). These results are not surprising, as farmers are more exposed to ticks and subsequent tick-bites than other individuals and therefore have more chances of contracting the disease.

In our present study the seroprevalence was found to be higher in older patients aged 41–55 years (2.94%, 95% CI 1.47–4.59) than younger ones aged 18–40 years (1.39%, 95% CI 0.44–2.38). This may be due to the fact that older individuals have more exposure time to livestock animals and because of their agriculture-related profession. Similar findings were reported by an earlier study in Iran (Mostafavi *et al.* 2017), where individuals with a longer duration of employment and exposure to livestock animals had significantly higher levels of seropositivity than other individuals. Additionally, a previous study from India reported that the risk of CCHF seropositivity was 3 times

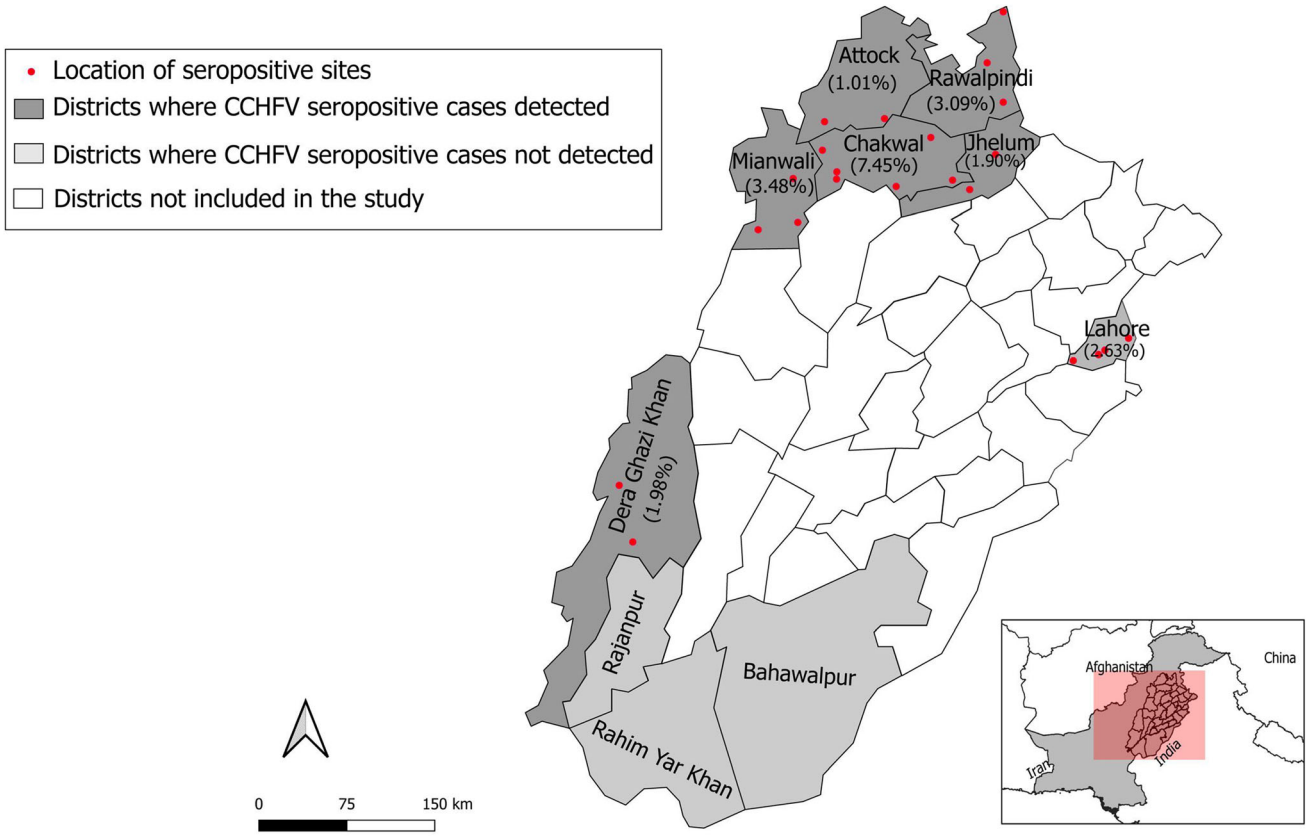

Fig. 1 Geographic distribution of CCHFV seropositive and seronegative districts of Punjab Province.

higher in older individuals (40–60 years) than in younger ones (< 40 years) (Mourya *et al.* 2019).

Our study has several limitations. As most of the individuals were illiterate and had a lack of exposure to scientific studies, they were hesitant to share the details of their work history or were unaware of any history of tick bites. Although they claimed to have a history of fever, due to the lack of access to a nearby appropriate diagnostic facility, they were not sure of any typical causative agent and had symptomatic treatments. As it evaluated samples originating from areas with and without a history of CCHF during a period when there was no epidemic, the study provides an assessment of seropositivity and its association with various categorical variables for individuals with a history of fever. Future studies with a larger cohort of individuals are required to ascertain the number of both clinical and sub-clinical forms of infection along with an exploration of ticks in a nearby setting.

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Compliance with Ethical Standards

Conflict of interest All authors declare no conflict of interest.

Animal and Human Rights Statement The protocols were approval vide letter no. IERB/129 dated September 26, 2016. Sampling and necessary procedures were conducted as per international ethical research guidelines.

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