A statistical approach to understand Crimean-Congo hemorrhagic fever prevalence in Pakistan

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ABSTRACT

Geographically, Pakistan is in the western part of south Asia at about 24-37 °N latitudes and 62-75 °E longitudes. Livestock and agriculture are two major sectors in Pakistan and play an important role in the country economy.

The tick infestation in livestock is not only devastating for animals and their products but also become the cause of transmission of pathogens into humans. Crimean Congo fever (CCHF) is a viral tick-borne fatal disease. The dissemination of ticks and amplification of Crimean Congo fever (CCHF) pathogen throughout the tick-animals-tick cycle, increases risk of transmission to humans many times. In Pakistan, cases are reported in all areas, particularly those areas which lie on the border to CCHF endemic countries. There is a high prevalence of CCHF in both Baluchistan and Khyber Pakhtunkhwa regions. Baluchistan is bordering with Afghanistan and Iran and Khyber Pakhtunkhwa with Afghanistan. Linear regression analysis revealed a positive significant association of high level of CCHF cases in livestock, with camels, goats and sheep. The literacy rate is negatively significantly corelated with the numbers of cases. Statistical analysis of border effect revealed a high positive significant correlation of CCHF prevalence in areas near to borders. Both Baluchistan and Khyber Pakhtunkhwa (KPK) has low literacy rate than other regions of Pakistan. Islamabad (capital city) has a higher literacy rate than all other regions but there is still a high CCHF prevalence. This is not only because of high population density but people from other regions, particularly from Baluchistan and KPK come here for animals selling or to seeking medical facilities in the large city hospitals. The study gives a proof that illiteracy and borders are the major respondent factors in the CCHF incidences and prevalence in an area. There is a need to raise awareness about ticks and tick-borne disease in the public and establishment of monitoring system across the borders to prevent the spread of CCHF virus.

Keywords: Crimean Congo hemorrhagic fever, Pakistan regions, literacy rate, livestock, border effect, linear regression.

Summary

Pakistan is an agricultural country and livestock is the important part in the people's socioeconomic life. Vector and vector-borne disease not only reduces the number of animals but also spreads disease into the human population.

Crimean Congo fever (CCHF) is a viral and vector-borne fatal disease that is spread by ticks' bite in both domestic animals and in human population. More the 60% of population in Pakistan are living in rural areas and the majority of the families in them are dependent on domestic animals' husbandry for livelihood. They come close to the animals and do not take precautionary measures due to lack of knowledge about tick-borne diseases. Our linear regression result showed that sheep, goats and camels have a positively significant association with high level of CCHF. The literacy rate is negatively significant associated with high level of CCHF cases which proves that there is unawareness in public about the disease spreading by ticks. The border effect result revealed that there is a highly significant correlation of CCHF prevalence in the regions that lie on the border. Analyses showed high prevalence of CCHF in Baluchistan and Khyber Pakhtunkhwa areas. In both these two regions of Pakistan, not only their literacy rate is low compared to other regions, unfortunately they also border Afghanistan and Iran where CCHF is endemic. There is free movement of people and transportation of animals across the border that is one main cause of the CCHF diffusion into Pakistan. Islamabad has a higher literacy rate than all other regions but there is still a high CCHF prevalence. This is not only because of high population density but people from other regions, particularly from border sites come here for animals selling or to seeking medical facilities in the large city hospitals. The authority must take steps to establish a proper monitoring system on the border region to check up the health conditions of population and the imported animals, especially when the situation is endemic in the adjacent area or a neighbor country. The government also needs to raise awareness in the people and educate the public about the CCHF disease and its spreading routs. Active surveillance system needs for accurate data collection that would be helpful in the CCHF control and prevention in other safe areas.

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1. Introduction

Geographically, Pakistan is in the western part of south Asia at about 24-37 °N latitudes and 62-75 °E longitudes (Sama et al., 2012). On the world map, it is situated on sub-tropical location, therefore, the temperature range there is from warm to hot in summer and from mild cool to cold season in winter. The country is differentiated into five regions due to temperature differences between them. The precipitation further divided the regions into arid, semi-arid humid and undifferentiated lands (Khan S et al., 2109).

Livestock and agriculture are very important sectors in Pakistan. They are adding a significant value into the country national gross domestic products (GDP). It covers about 45% employments of the labor market and play a vital role in Pakistan's economy (Kakar RA et al., 2009; Rehman A et al., 2017).
In 2017 census, according to Pakistan Bureau of Statistics (PBS) the population was 207,774,520 and increases by approximately 2% every year.
In Pakistan, more than 8.0 million of the population are rural families which depend on animal husbandry for earning their livelihood. Livestock husbandry is not only providing healthy food to the people and alleviating poverty, but domestic animals are also used for fetching water, loading woods, carrying foliage for livestock etc. Donkey, mule, horse and camel are cheap means for travelling, transporting goods and for other commercial activities (Khan et al.,2015).

Livestock and agriculture are an important socioeconomic part in any developing country especially a country like Pakistan where much of the population, about more than 70% are rural and their main source of income is rearing of animals (Rehman A et al., 2017). There are 175 million livestock animals in the country (Rehman K et al., 2018).

The rural areas where people's livelihood is dependent on rearing domestic ruminants, the vectors and vector-borne diseases are a serious problem for them. The vector ticks ecological and geographical distribution in Pakistan has greatly affected the livestock sector (Rehman A et al., 2019). The tick infestation in livestock is not only devastating in animals and their

products such as meat, milk, wool and hides but also become a source of transmitting pathogens into humans (Karim K et al., 2017).

In livestock, Crimean Congo fever is one of the most prevalent disease in Pakistan. In 1976, the first CCHF case was identified in the Center General Hospital, Rawalpindi city. (Yaqub T et al., 2019). Sporadic outbreaks in people has been reported from different areas in Pakistan, since the year 2000 (Khan AU et al., 2017).

Every year, from 5 to more than 50 CCHF cases happen due to virus spillover from animals to human population. In 2016, there were 50 cases and 19 death reported in Pakistan (Wahid B et al., 2019). In 2018, there were 63 CCHF laboratory confirmed cases and the number further increased to 75 CCHF in 2019 (NIH, FDSRU. 2020).

The literacy rate is very low and mostly people are illiterate in the rural areas of Pakistan. The average literacy rate in KPK is 53% and in Baluchistan is 44% as compared to Punjab and Sindh where average literacy rate is above 60% while it is up to 90% in Islamabad (Rehman A et al., 2016). These people from rural areas are totally unaware about vector and vector-borne diseases. They do not know about diseases and pathogens that are spreading by ticks to both humans and animals. Vectors and their borne diseases are a serious problem in the rural population as they are also far away from the reach of healthcare facilities. This lack of knowledge due to illiteracy and being far away from medicine facilities such as vaccination due to poverty, they are highly prone to the zoonotic diseases (Atif M et al., 2017; Sajjidi NA).

Nomadic lifestyle in Pakistan is another source of CCHF virus dissemination from one area to another area. There are tribal areas in Pakistan where some people have nomadic lifestyle, shifting from place to place according to the change of weather wherever food and water are easily available for them. These nomadic people sometime become a high risk in the spreading of virus. They move together with their ruminants and along with them take the tick infested and viraemic animals from endemic area into the new area which was before free from CCHF virus (Ahmad T et al., 2016).

CCHF virus vectors are evolved to the extreme, arid, semiarid, hot and dry environment. In previous studies it is well documented that high temperature, moisture and precipitation are the predictors for spreading CCHF virus. The humid climate with little rainfall and presence of reservoir animals in the area increase the chance of ticks' survival and life cycle (Messina JP et al., 2015).

The change in climate and environment, movement of animals across the borders and tick propagation and infestation are the factors in the spreading of CCHF virus in an area. The climate and environment of Pakistan, especially in south and west are highly favorable for tick propagation and survival. The country has little rainfall, warm weather from March to November and mild winters, these factors increases the prevalence of ticks *Hyalomma* in the region (Ahmad T et al., 2016; Rehman A et al., 2017).

2. Aim of Study

The purpose of this study is to see statistically that how some areas of Pakistan are hit with a high prevalence of CCHF; it examines the effect of human and animal population and geographical position of areas in CCHF outbreaks. The study would be helpful in the understanding of CCFH virus transmission and in taking precautionary measurements for stopping it from further spreading in the population.

Addressed questions:

- *Why there is high CCHF prevalence in an area where population density is low while another area having high population density has low CCHF prevalence?*
- *Apart from climate and environmental factors what are the other explanatory variables which are causes of high incidences of CCHF in an area?*
- *In the spreading of CCHF virus, is there an across border movement effect in the area?*

3. Background

3.1 Crimean Congo Hemorrhagic Fever (CCHF) Virus

Blood feeding arthropods are widespread in different parts of the world and transmitting a wide range of pathogenic bacteria, protozoa, rickettsia, spirochetes, and viruses (Farooqi HS et al.,2017, Saleem M et al.,2016)

Vectors like ticks are transmitting infectious pathogens into both livestock animals and to humans. Ticks are responsible for spilling over many bacterial and viral pathogens from their wild animals' reservoirs such as rodents, hedgehogs, mice etc., to domestic animals like camels, cattle, buffalos, sheep, goats, horses, donkey/mule etc. This emerging virus then spillover to the human population from domestic animals (Kemp A et al., 2014).

Crimean-Congo hemorrhagic fever (CCHF) is a viral infection, transmitted by tick bite or through contact with contaminated blood, secretions and tissues of animals or patients. The disease is endemic in many regions of the world such as Asia, Africa, middle East and southern and eastern Europe (Leblebicioglu H et al., 2015, Saleem M et al., 2016, Wahid B et al.,2019, Ahmed A et al., 2018, Oluwayelu D et al.,2020).

Crimean-Congo hemorrhagic fever virus is a negative sense RNA virus covered by spiky glycoprotein making size up to 100 nm. It belongs to genus *Nairovirus* and family *Bunyaviridae*. CCHF is a zoonotic disease transmitted by hard ticks belong to *Ixodidae* family. Many *Ixodid* tick species are found transmitting the virus, but genus *Hyalomma* ticks are the basic vectors spreading CCHF virus into the host animals. First CCHF incidence was found in the Crimean in 1944 and later the causative agent isolated in Congo in 1959. The infectious virus is an emerging pathogen across the world. It causes severe viral hemorrhagic fever outbreaks and has a fatality rate up to 40%. (Al-Abri SS et al.,2017; Ahmad T et al., 2016; Wahid B et al.,2019.

There are 6 different strains of the CCHF virus, i.e., Asia-1, Asia-2, African-1, African-2, African-3, Eru-1, Eru-2. (Abbas et al., 2015. Who.2020).

The CCHF virus RNA genome is composed of three segments, small (S) 20, medium (M) 31 and large (L) 22 nucleotide which is covered by a lipid bilayer membrane.

These segments S, M and L of CCHF virus RNA express into three different proteins such as nucleocapsid, glycoprotein and polymerase respectfully (Mishra AK et al., 2020; Deyde VM et al., 2006; Chinikar S et al., 2010).

The infected ticks transmit CCHF virus in both the transstadial as well as transovarial way into the host. The horizontal transmission of CCHF virus occurs during blood feeding (biting) and vertically take place through passing the virus into their eggs. In this way the spread of virus is amplified many times (Wahid B et al.,2019; Kasi KK et al., 2019 & 2020).

The virus remains in the blood of animals for a week and become viraemic without any symptoms. The animals play a major role in the spreading of virus as it circulates between ticks and animals. The virus is found in many genera of ticks but genus *Hyalomma* is the basic reservoir vector for transmission. *Hyalomma asiaticum* and *Hyalomma marginatum* are known vectors in Asia and Europe respectively (Leblebicioglu H et al.,2015; Al-Abri SS et al.,2017).

3.2 Clinical manifestation

People are infected either by direct tick biting or through contaminated blood, tissues or secretions from infected animals or infected persons. Usually the incubation period ranges from 1 to 14 days which depends how the virus is transmitted; it takes 1-3 days by tick bites and 5-13 days through contact with infected blood, sections or tissues. The disease symptoms include fever, nausea, vomiting, diarrhea, abdominal pain, headache, backache, pain in neck, muscle ache, sore eyes and light sensitivity. After a few days, the patient gets agitation and then depression. The patient has pain in the upper right body due to hepatomegaly (enlargement of lever); have tachycardia, petechial rash, ecchymoses and other hemorrhagic on internal mucosal surfaces). Final liver failure and kidney impermeant causes death of the patient (Who.2020; Saleem M et al., 2016, Wahid B et al., 2019, Zohaib A et al., 2020).

3.3 Epidemiology

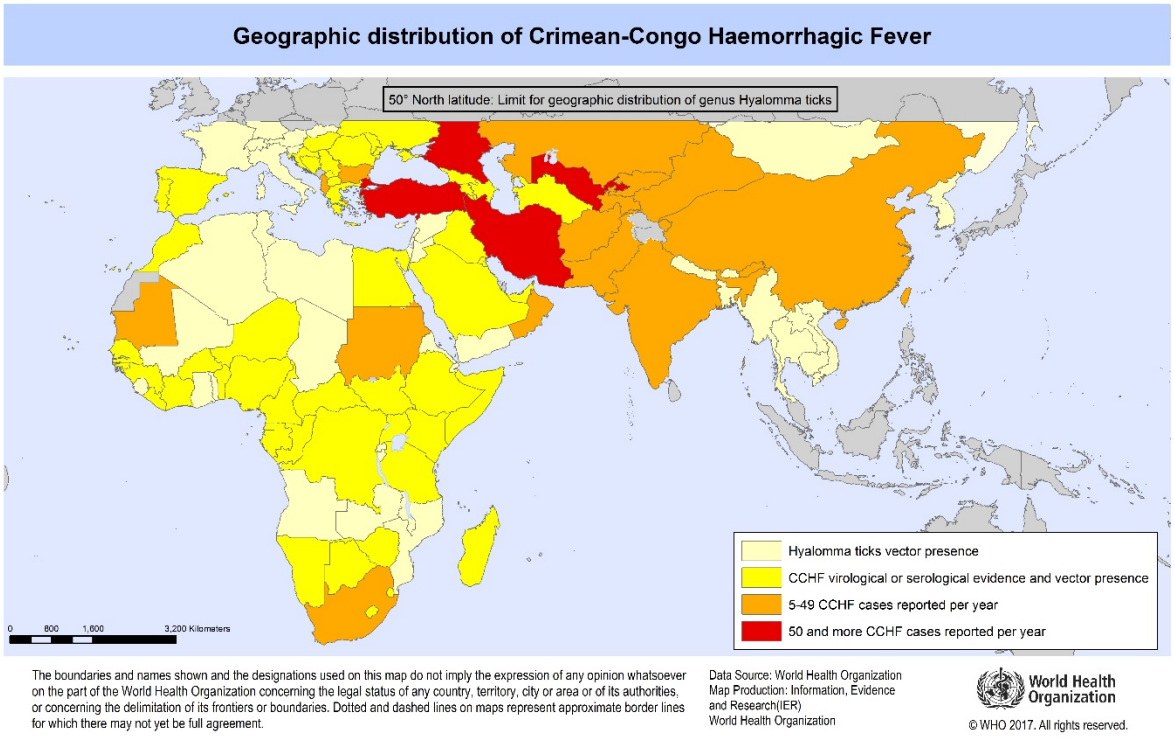

Fig:1. The CCHFV prevalence in the different parts of world. Source: WHO link:

https://www.who.int/health-topics/crimean-congo-haemorrhagic-fever/#tab=tab_1

(Note the map is adopted from WHO website at 7:00pm in 19th April,2020)

The geographical range of CCHF virus is across the world, in more than 30 countries CCHF disease is identified. The first CCHF case was identified in Crimean in 1944, the former Soviet Union. The causative agent was not known until 1956 and then it was diagnosed in the hemorrhagic patient in Democratic Republic of Congo (Leblebicioglu H et al., 2015). Later on, the virus spread to other regions of the world such as Russia, Serbia, Kosovo, Hungary, Bulgaria, Turkey, China, Pakistan, Iraq and United Arab Emirates (Okely M et al., 2020).

There are sporadic outbreaks in parts of Africa, Southeast Europe, Middle east and Asia; it is endemic in some areas of Turkey, Iran, Afghanistan and Pakistan (ECDC.2020; Saleem M et al., 2016).

Serological based analysis of the population from India, Turkey, Bulgaria, Kosovo and Greece found positives of 0.5%, 10-19.6%, 3.7%, 9.3%, 4.9% respectively (Shahid MF et al., 2020) Seroprevalence in Serbia 4%, Hungary 2.8%, Georgia 2.8%, Russian Federation 11.1% and

Kazakhstan 12.7% (Monsalve-Arteaga L et al., 2020) The percentage of seroprevalence from different regions of the world shows the level of CCHF incidences in that country and its epidemiology in the world.

Hot and dry environment, arid or semiarid climate land, scanty rainfall and humid condition are the indicators for tick prevalence and infestation in the area (Messina JP et al., 2015).

The southeastern Europe, western Mediterranean and northwest African countries climate and environment is suitable for ticks and CCHF virus persistence. In southeast Europe, especially Turkey and Bulgaria are highly favorable for tick survival, that is why there is high seroprevalence of CCHF virus in people from these areas. (Messina JP et al., 2015; Okely M et al., 2020). From 1953-2008 in north-east Bulgaria, 1568 CCHF incidences are reported by WHO (WHO.2008; Hussain B et al; 2016).)

Centers for Disease Control and Prevention (CDC) has determined that CCHF virus is a biosecurity threat. WHO has declared that among eight important emerging pathogens, CCHF virus is one of the them (Monsalve-Arteaga L et al., 2020)

3.4 Phylogeny across the border

The CCHF virus has large diversity as the dissemination easily take place across the country borders through tick infested viraemic animals and infected humans. The CCHF virus genotype isolated in Pakistan is Aisa-1 with four sub-strains. These CCHF virus strains phylogenetically identified 90 to 100% similar with the neighbor countries (Afghanistan and Iran) strains (Wahid B et al., 2019).

Turkey is an import region on the world map, a bridge between east and west where the movement of trans-border viraemic animals spread ticks and CCHF virus. The phylogenetic study shows that different geographical CCHFV strains have the same origin from a gene pool. The different types of CCHF strains have evolved through reassortment or mutation after the cross-border movement of infected humans and animals. It is found that even migratory birds are travelling with infected ticks across the county (Leblebicioglu H et al., 2015; Khurshid A et al., 2015; Saleem M et al., 2016; Zohaib A et al., 2015).

3.5 Diagnosis

The early detection of CCHF virus is only possible through rRT-PCR assay. It is expensive but a very sensitive and reliable diagnostic tool (Schnittger et al., 2004; Garcia S et al., 2010). Other methods include immunofluorescent and antigen- capture ELISA assay is used to detect antigens of CCHF or antibodies against CCHF virus in the patient blood (Tang Q et al., 2003). Other techniques to diagnose CCHF virus is the sample inoculation into mice and cell culture techniques are also a useful method (Chinikar S et al., 2010; Logan TM.,1989).

3.6 Treatment and Vaccination

If there is a suspected CCHF patient and defined CCHF case, then Ribavirin treatment must be given. Ribavirin is still one of the most common used anti-CCHF virus medicine in treatment. Using plasma with anti-CCHF virus antibodies is found effective way of treatment for CCHF patient. The FDA has also approved the synergist effect of chloroquine or chlorpromazine with Ribavirin in CCHF patient treatment (Wahid B et al; 2019; Al-Abri SS et al., 2017)

The CCHF is highly prevalent in animals because they have no symptoms of infection. Use of acaricide for tick eradication in animals is useful and it would be better to isolate viraemic animals for couple of weeks before sacrificing them. Unfortunately, there is no vaccine available either for human or for animals against CCHF infection (Hussain B et al; 2016).

3.7 Precautionary measurements

Taking precautionary measurement to control the spreading of CCHF virus, the basic is to educate people and raise awareness in them specially in the exposed groups such as agrarian man, veterinarians and health providers.

People who are close working with animals should use light color clothes so that ticks could be seen easily to remove. Visiting livestock or habitats where ticks is prevalent, repellents diethyltoluamide (DEET) to skin should be used to avoid tick biting (ECDC.2020; Yadav et al; 2015). In case of a tick bite, wash the area with soap and contact the health care center. Animals should not be handled without gloves, especially animals which come from tick's endemic regions. The butchers need to use protective measures, do not slaughter without using glove (Oncu et al;2013). Blood and waste of animals must be disposed hygienical to avoid transmission of CCHF virus through contact. The health care providers are at high risk getting CCHF through contact with patient blood, fluids or secretion. They need more care providing

medical assistance to the CCHF patient. Antiviral drug Ribavirin can be taken as prophylaxis in case of exposure (Leblebicioglu H et al., 2015; Al-Abri SS et al.,2017). Ribavirin has been used for treatment of CCHF patients, but its efficacy is not so clear. Many research articles reports tell that it is the only drug for treatment and reduce the fatality rate (Al-Abri SS et al., 2017; ECDC.2020).

4. Previous Studies

Leblebicioglu H et al., (2015) have reported that across the border travelling or transporting of infected animals and migration of birds with infested ticks are playing a role in the spreading of CCHF virus. The trans-border free movement of infected human and animals plays a major role in the spreading of disease. Turkey and Iran have important geographical positions between east and west. Unfortunately, both countries are CCHF endemic and bordering between east and west. There is free trading of livestock across the border that facilitates diffusion of CCHF virus from one county to another country.

Khurshed A et al., (2015) carried-out a study on CCHF suspected patients at the NIH in Pakistan. One hundred samples were collected for anti-CCHF-IgM and CCHF virus RNA analysis. They used ELISA and PCR methods for antibodies and RNA detection respectively. Samples result was 49% positive for CCHF virus. 10 samples were found positive for IgM antibodies, 14 for CCHF virus RNA while 25 samples were positive with both ELISA and PCR. All positive patients were Pastoralists from Baluchistan- province. 39 PCR positive CCHF virus nucleotides sequencing revealed that 7 of them belongs to a neighbor country clade. The alignments with representative strains from GenBank showed close similarity with strains from Afghanistan. There was 4% diversity in these 7 clades of strain from the neighbor country. The study identified that all strains clustered with the strains from Pakistan, Iran and Afghanistan with Aisa-1 type of strain. In 1976, after identification of the first patient of Crimean Congo hemorrhagic, 14 sporadic outbreaks have hit different areas of Pakistan; especially Baluchistan and Khyber Pakhtunkhwa which shares border with Afghanistan and Iran (Khurshed A et al., 2015).

Khushal Khan Kasi et al., (2020) conducted a comprehensive epidemiological study for CCHF prevalence in small ruminants in Baluchistan province. They used a cross-sectional study for examination of CCHF in sheep and goat to diagnose CCHF seroprevalence in them. Every year, the highest numbers of cases are reported from Baluchistan with fatality rate of 20%. For serological test they collected 800 blood samples from sheep and 800 from goat at different animal farms in different areas in Baluchistan. The ELISA technique was used to diagnose CCHF-Ig specific antibodies. Seropositivity of CCHF in sheep was 19% (146 out of 800 sheep samples) and 5% in goat (37 out of 800 goat samples). Their result showed that blood sample of 81 farms out of 160 were positive with 51% seroprevalence in the region. Further they also statistically analyzed that there was no relation between education and CCHF infection. Using the questionnaire method, they found that 60% had knowledge while 40% were unaware about CCHF disease. This seropositivity in animal and the high percentage of seroprevalence from animal farms in different areas is a threat and main source of transmission infection to humans.

Similarly, Ali Zohaib et al., (2015) study also has showed a significantly higher seroprevalence for CCHF in animals and humans from Baluchistan and KPK than other regions of Pakistan. Using ELISA technique for blood samples they found 5.7% seroprevalence of CCHF in Baluchistan, 4.7% in KPK, 2.7 in Punjab and 1.1% in Sindh human population while the livestock seroprevalence of CCHF in these regions found 59.3%, 52.4%, 24.7% and 16.2% respectively. Further, their result showed very high prevalence of CCHF in camels compared to other livestock animals. It is clear, there is a high number of camels in Baluchistan.

Baluchistan province, the fact that it shares its border with Afghanistan and Iran is the likely cause of CCHF prevalence. There is free movement across the border and people bring animals for trading. To test this hypothesis Asad Mustafa Karim et al., (2017) conducted sera analysis of animals that were imported from Afghanistan and Iran. They collected blood samples randomly from 21 imported animals and tested for anti-CCHF virus immunoglobulin through ELISA technique. They diagnosed 13 CCHF positive out of 21 samples, which makes 62% seroprevalence of CCHF in transported animals from neighbor countries. To strengthen their hypnosis, they examined data from CCHF patients demographically and found 58% of them were belonging to the neighbor counties. These trade animals that might be CCHF virus positive are then taken to other regions in the country and become a serious risk of CCHF outbreak. They further studied the possible time for CCHF incidence during the year. It was found that usually death cases increase near the time of festival when people are buying animals for Eid-u-Adha (religious festival). During this period, selling the transportation with livestock

is increased from one area to another even across the border. The movement of infected animals from rural to urban areas brings people close to contact to them and increases chances of getting CCHF infection.

Tariq Abbas et al., (2015) has analyzed CCHF cases in 2013 data from INH and identified hotspots in Pakistan regions. Using spatial scan statistic, they found that there are two important CCHF clustered areas in Pakistan. Baluchistan province found is the larger CCHF clustered area that lies on the border of Afghanistan and Iran. Another clustered area was Rawalpindi, the twin city to Islamabad (capital of Pakistan). There is trading of animals and animals' products across the border and then from Baluchistan they are supplied to other cities such as Rawalpindi, Islamabad. During time of Eid-ul-Adha festival when animal's transportation increases, the risk of CCHF virus dissemination also increases.

5. Methods

The dataset about the prevalence of Crimean Congo hemorrhagic fever cases was retrieved from the National Institute of Health (NIH), Islamabad and field of epidemiology & disease surveillance division Pakistan website. NIH and field of epidemiology & disease surveillance division is working in cooperation with the regional office of the World Health Organization (WHO), Pakistan. The data consisted of total numbers of human CCHF NIH laboratory confirmed cases from the different country regions under the period from 2014 to August 2019. Literacy rate is acquired from the Pakistan economic survey 2015-16, finance division, government of Pakistan, Islamabad. Information of livestock was acquired from the government of Pakistan Statistic Bureau (PSB), provincial-wise livestock senses 2006. Livestock information of Azad Jammu & Kashmir collected from the Bureau of Statistics of AJ & Kashmir. Previous reports were used to complete the missing data; the population was extrapolated from the linear growth ratio, livestock to 2006 and human to 2019. Livestock animals were taken per million for the number of cases as a response variable. The linear regression statistics was used to find the relationships of explanatory variable to the human

incidences in the region. To see the border effect in regions with CCHF prevalence, in linear model analysis depending on the neighboring country's CCHF endemicity the border was considered yes (y) or no (n) if no endemicity. For significant variable, null hypothesis was rejected with p-value < 0.05. We used R and R-Studio software to analyze the data. R version 3.6.0 (2019-04-26) – "Linear regression and linear model plot". Copyright (C) 2019 The R Foundation for Statistical Computing.

6. Results

6.1 Statistical Analyses

Linear regression method was used to explore data for the risk factors associated with CCHF incidences in the different regions in Pakistan. Different livestock animals were analyzed as explanatory variables for the number of CCHF cases in regions. Confounding factor was evaluated through looking at the border that how it effects on the region for CCHF prevalence. Camels, goat and sheep were found with positive significant association to the CCHF incidences. We reject the null hypotheses as p-value is less than 0.05 ($p < 0.05$). The result show positive significant correlation of CCHF cases with number of camels, sheep and goat. But we found a non-significant association ($p< 0.05$) of cattle, buffalo and donkey/mule/horse for CCHF cases. To evaluate the effect of different literacy rate in the different regions, we found a negative significant association of literacy rate with the number of incidences in the regions with p-value < 0.03. To reveal the confounding factor, we statically analyzed the border effect on the region through effect plot analysis. We found p-value 0.003, shows that the border effect has highly significant association with prevalence of CCHF in the regions.

Table:1. Statistical linear regression analyses of explanatory variable for Crimean-Congo hemorrhagic fever virus cases and prevalence

Variables	Correlation Coefficients	Significant P-value (p< 0.05)
Cattle	0.000003935	0.303
Buffalo	0.000002378	0.435
Donkey/mule	0.00002407	0.358
Camel	0.00025775	0.00482
Sheep	0.0000004643	0.000229
Goat		0.0464
Population size	0.0000004643	0.336
Border effect	78.355	0.00361
time	7.8983	0.00477
Literacy rate	-2.4364	0.0365

6.2 Result Analysis

Under period 2014-2019, there are a total of 314 NIH laboratory confirmed CCHF cases reported. The highest number is from Baluchistan region with 122 cases (39%) then followed by Punjab 79 cases (25%), Sindh 50 cases (16%), KPK 41 (13%), Islamabad 16 (5%), AJ Kashmir 1 (0%) and 16 cases (2%) from FATA8 (fig.2).

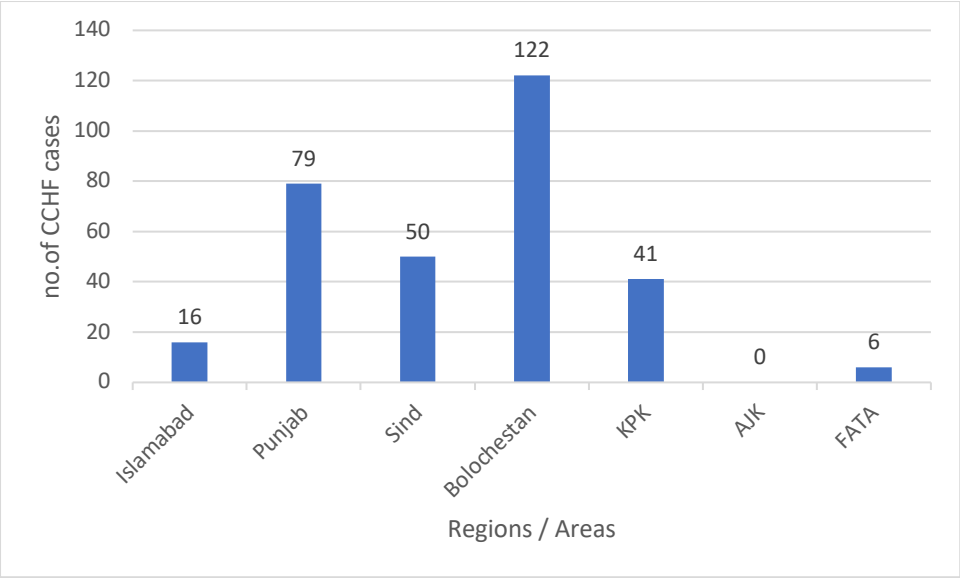

Fig:2. National institute of health sciences (NIH) lab confirmed cases by province/region in Pakistan from 2014 to August 2019(n=314).

We analyzed the number of CCHF cases in different regions with livestock animals and found significant association with camels, sheep and goat (fig 2,3 & 4).

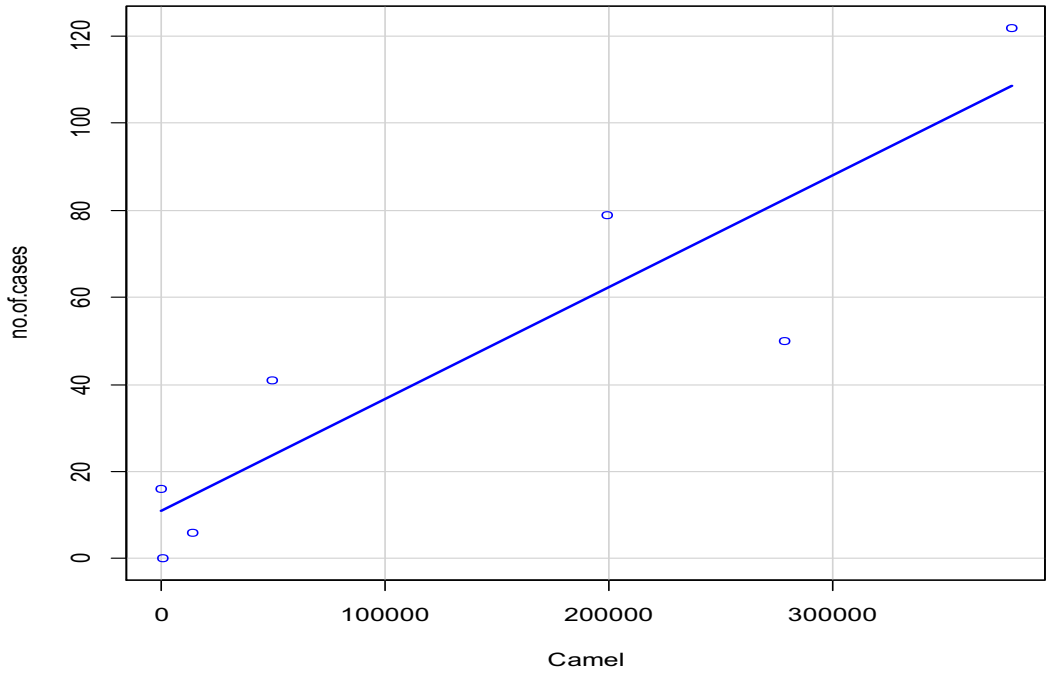

Fig:2. The scatterplot shows a positive significant correlation of camels with number of CCHF cases in the different regions in Pakistan.

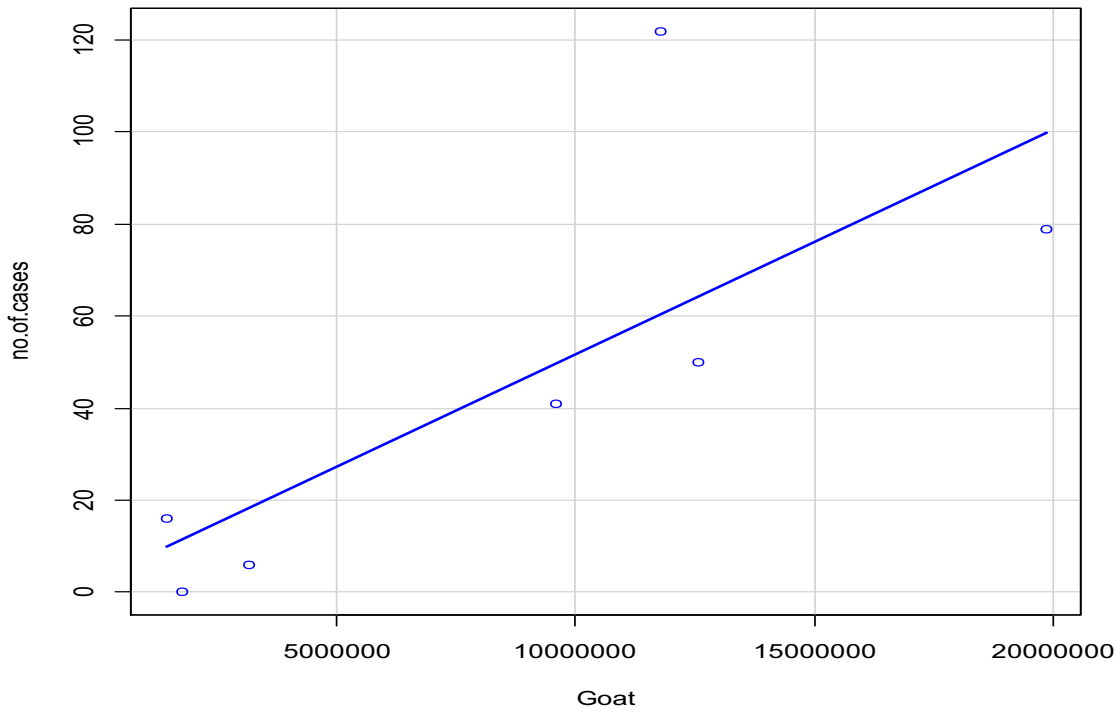

Fig:3. The scatterplot shows a positive significant correlation of camels with number of CCHF cases in the different regions in Pakistan.

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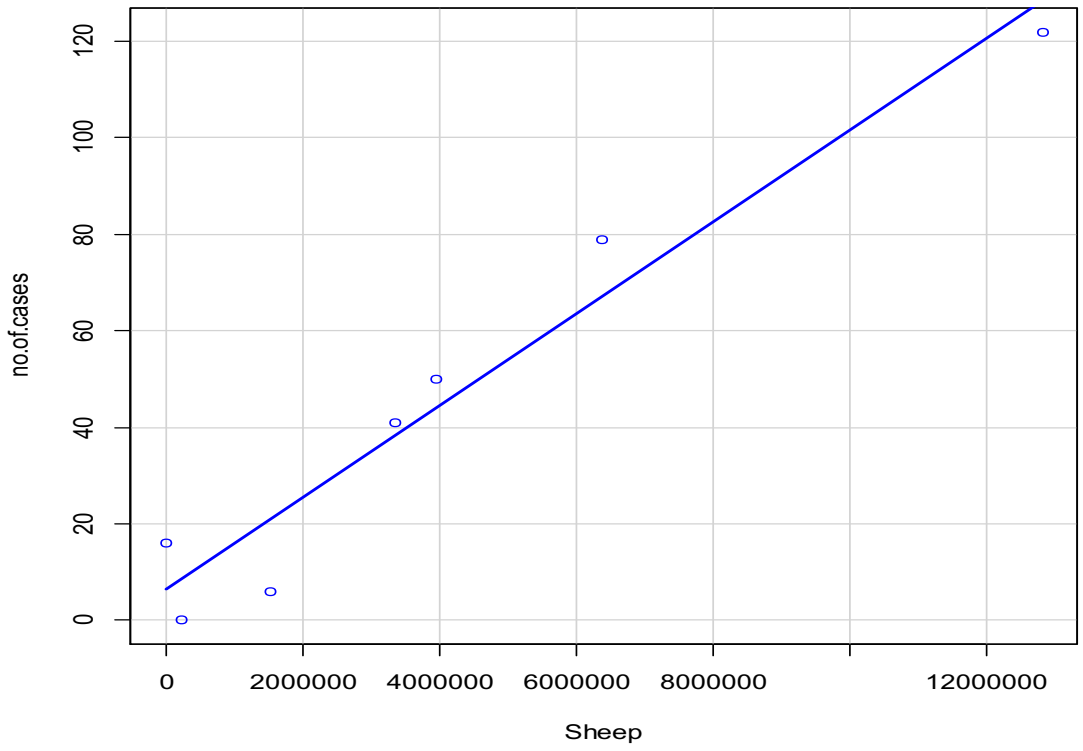

Fig:4. The scatterplot shows a positive significant correlation of sheep with number of CCHF cases in the different regions in Pakistan.

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The y-axis shows respondent, case numbers against the explanatory variable on x-axis. Baluchistan has the highest number of camel and sheep then followed by Punjab and Sindh.

Livestock goats found significant for high number of CCHF cases, they are in high numbers in Punjab, followed by Sind and Baluchistan region.

As expected, we found negative significant relationship between literacy rate and the number of CCHF cases with p value 0.03 (fig.5).

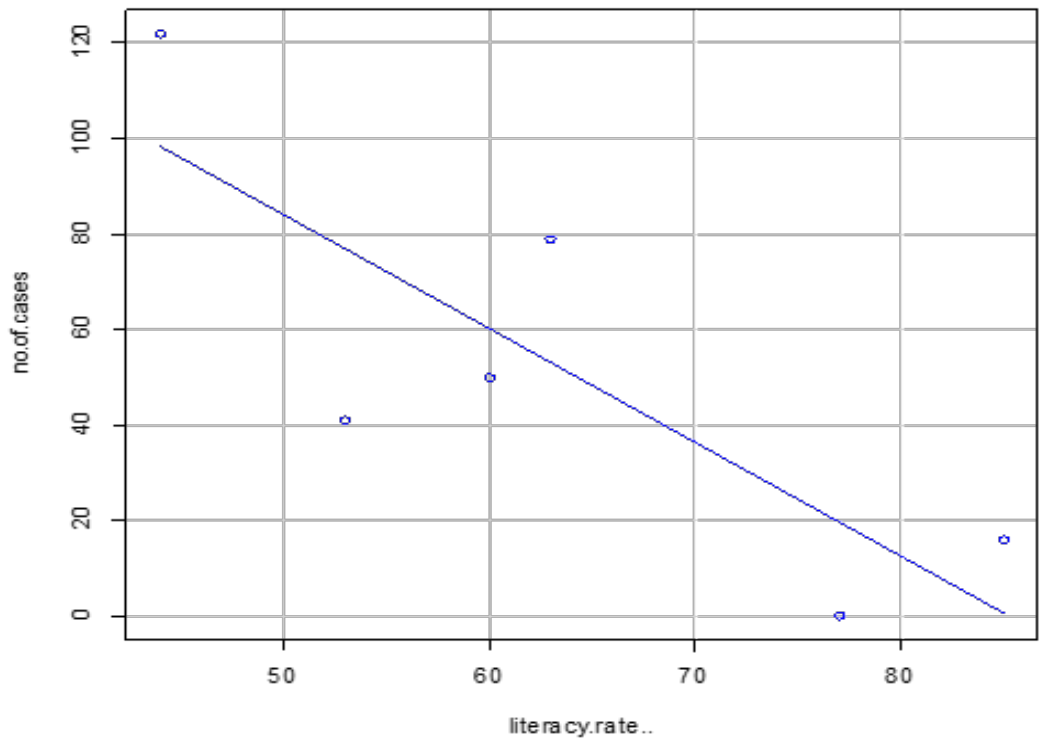

Fig:5. The scatterplot shows a negative significant correlation of literacy with number of CCHF cases in the different regions in Pakistan.

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As we see the literacy rate of KPK and Baluchistan is lower than Punjab and Sindh and so, there is a high prevalence of CCHF in these first two regions. But on the other hand, in capital city Islamabad, where the average literacy rate is approximately 85%, CCHF prevalence is near to the numbers in Baluchistan, where the literacy rates are lower than in Islamabad.

In fact, it is affected by confounding factors, possibly by population density or other unknown factors.

To see the border effect on two regions for high prevalence of CCHF, we used effect plot analysis (fig.6).

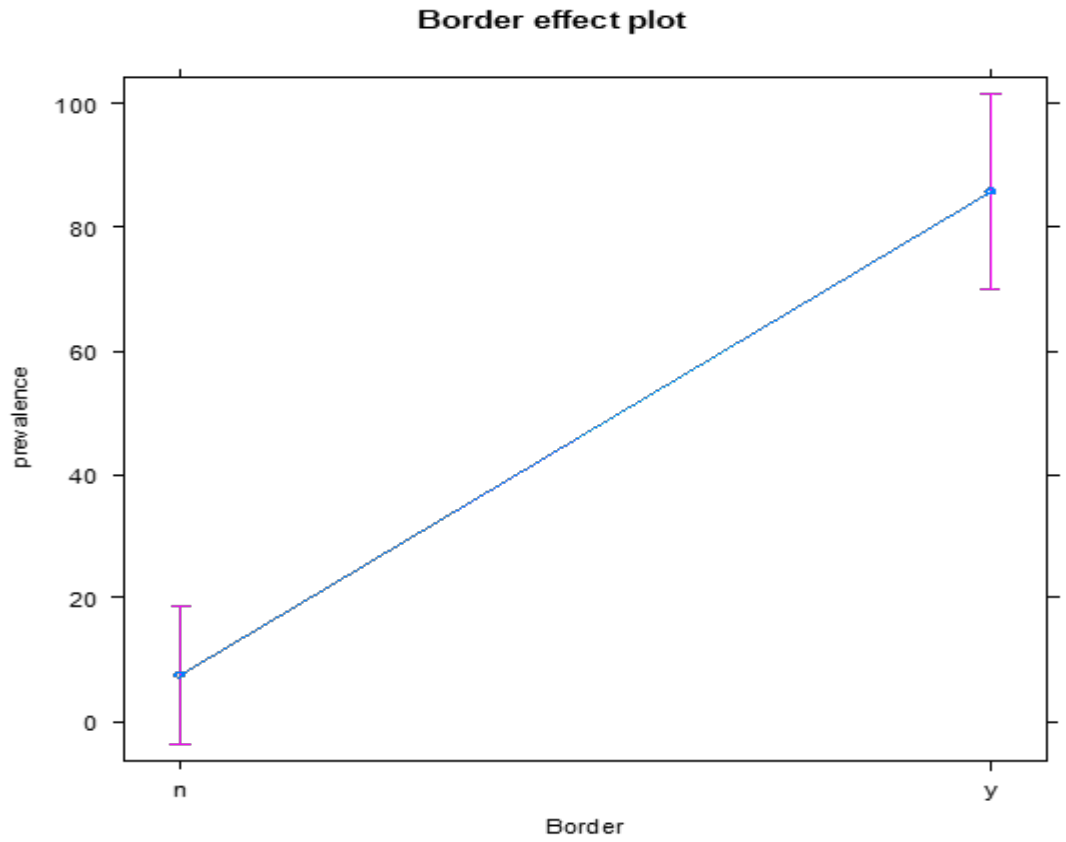

Fig:6. The figure showing a positive correlation between border effect (border no= n & border yes= y) and the prevalence of CCHF in area.

We found positive significant border effect on the regions close to neighbor countries Afghanistan and Iran with p-value 0.003. Effect plot analysis revealed an important border associated risk factor for the increased number of CCHF outbreaks in the country. This positive significant correlation between border and high level of CCHF prevalence is attributed through high number of camels, sheep and goat in the region.

Then we looked for CCHF incidences in Pakistan with time. The effect plot showing a positive significant correlation of CCHF cases with time. It has been reported that there were 34 case in 2014, 50 in 2016, 63 in 2018, and 75 in 2019. It indicates that there is an increase in the number of CCHF cases with time in the country. (fig.7).

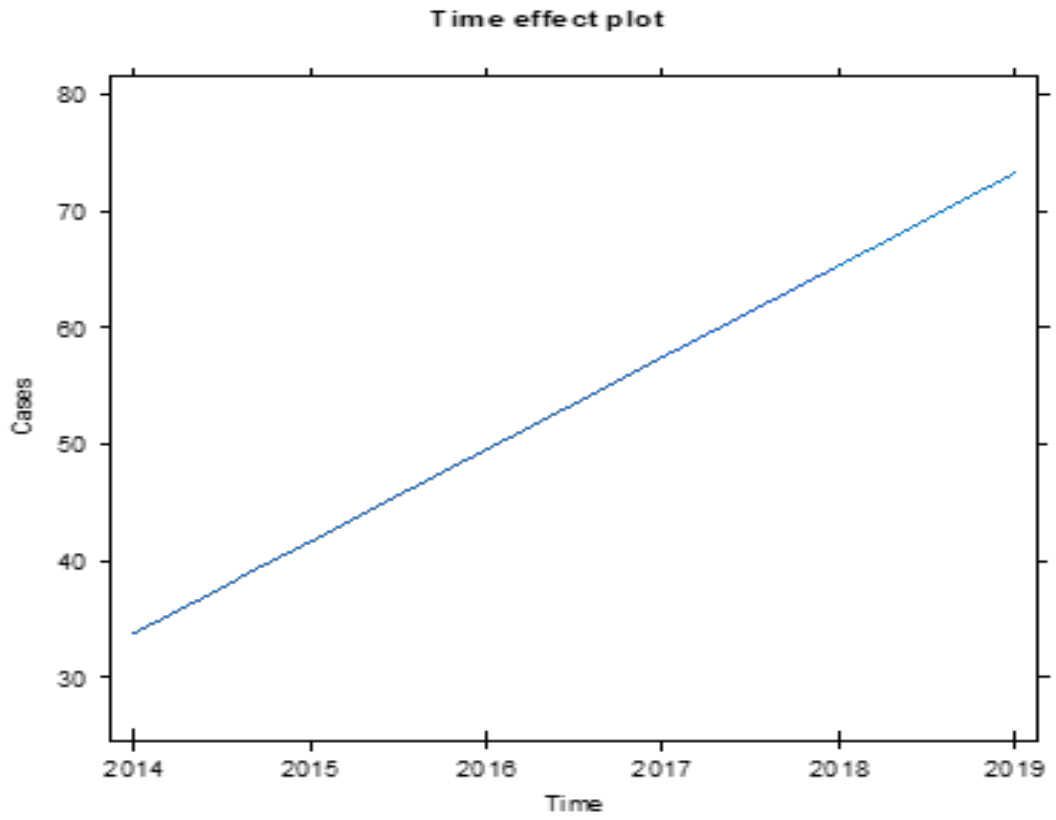

Fig:7. The time effect plot shows an increase number of cases in Pakistan with time.

Many previous reports have documented that western and northwest region of Pakistan are hit with high number of CCHF outbreaks. Although high number of incidences are also reported from other parts of the country, but regions Baluchistan and Islamabad have the highest level of prevalence in the area (fig.8). Both regions population size is very low compared to Punjab and Sindh. But the population density of Islamabad is very high compared to other regions (fig.9).

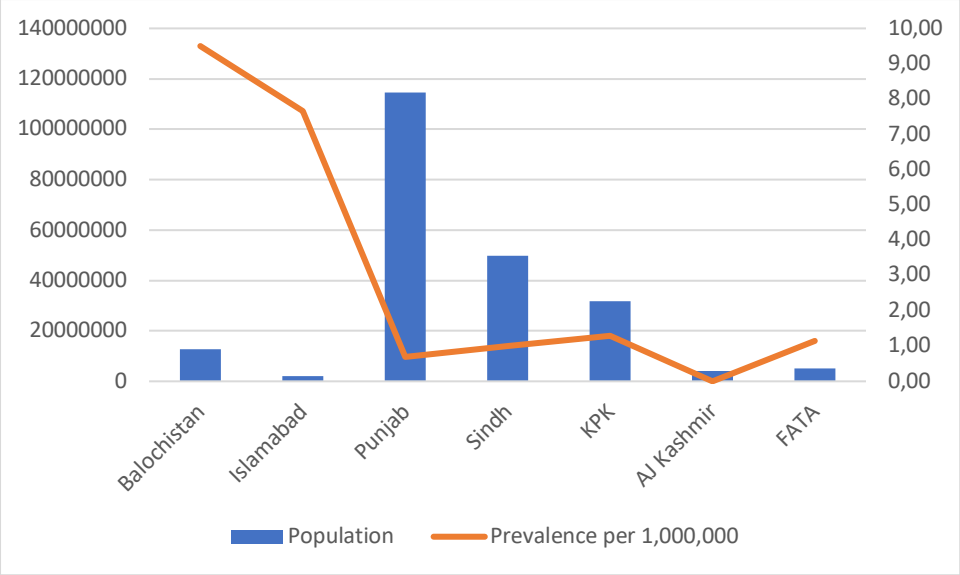

Fig:8. The figure shows the prevalence of CCHF per 1.000.000 in the population in the regions of Pakistan.

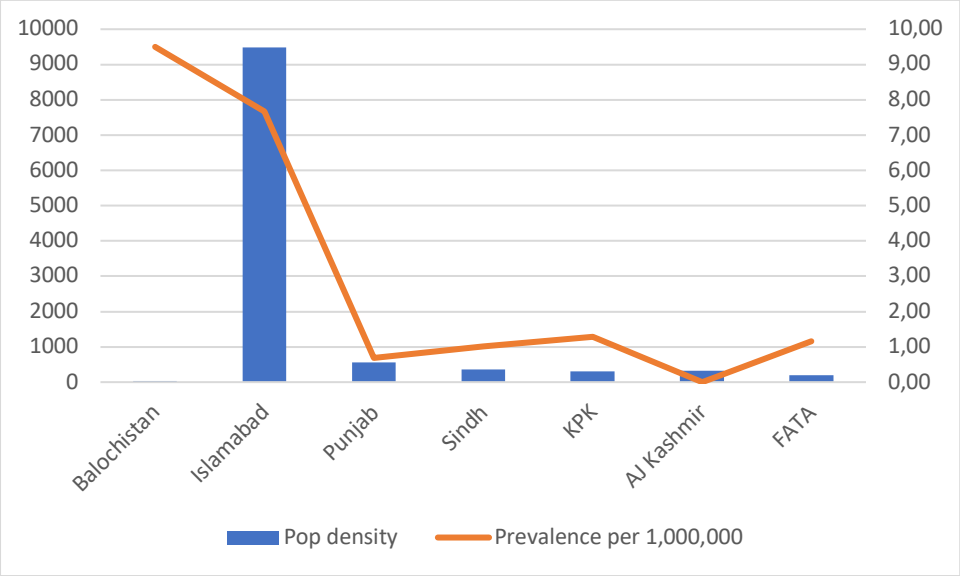

Fig:9. The figure shows the population density and the prevalence of CHHF per 1.000.000 in the population in the regions of Pakistan.

These high prevalent regions lie on border with Afghanistan and Iran which both are endemic countries (Ahmad A et al., 2015). Islamabad is the capital city of Pakistan, the total registered number of CCHF cases (period 2014-2019) are 16 which are very low compared to Punjab 79 cases, Sindh 50 cases and Baluchistan 112 cases. But the level of CCHF prevalence in Islamabad is very high and is near to the level in Baluchistan region. Because there is a big difference in these two regions area and the population density that change the pattern of CCHF prevalence from other regions in the country.

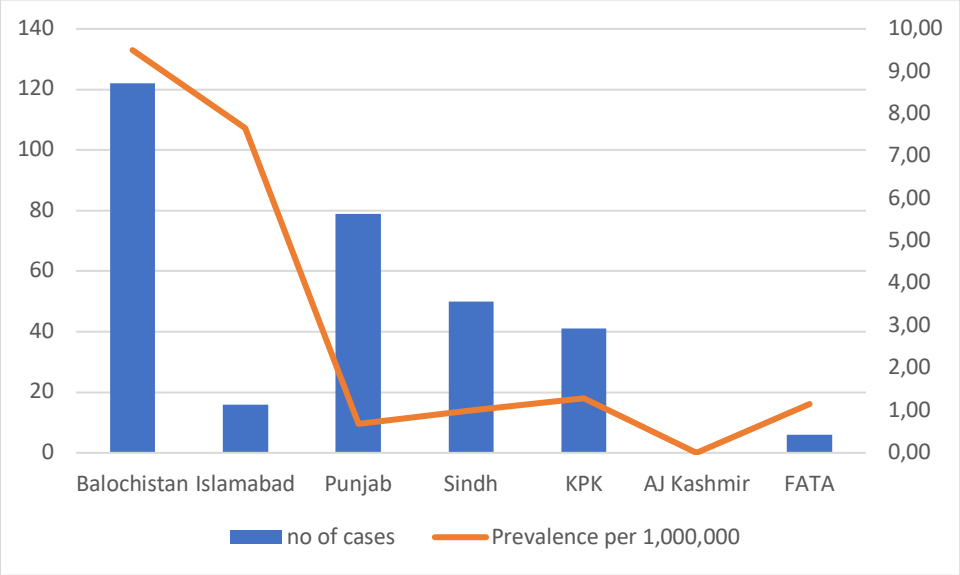

Fig:10. The figure shows the number of CCHF cases and the prevalence per 1.000.000 in the population in the regions of Pakistan.

Muhammad Abbas et al has found that Baluchistan and Rawalpindi are the two major area of CCHF clustered, the first one bordering Sindh province while the other one is the twin city to Islamabad.

7. Discussion

The data from NIH that covers a 6-year period shows high numbers of incidences during spring and autumn months (NIH.2020). This seasonal and climate effect on the pattern of incidences, a high peak from March to May and then again from August to September is supporting all previous reports. There is an impact of changing weather on CCHF prevalence in the region. The warm autumn, mild winter and little rainfall in summer are key factors in the tick propagation and infestation (Khurshid A et al., 2015).

The unawareness about CCHF virus and the vectors that are spreading this disease is due to the illiteracy of the people. The literacy rate of Islamabad, Baluchistan, Punjab, Sindh, KPK and AJ Kashmir is 85%, 44%, 63%, 60%, 53% and 77% respectively (Reman A et al., 2015). The significant relationship of low literacy with high number of CCHF cases show lack of knowledge of people about tick borne disease. More than half the country population are rural, and they are unaware about infections and disease, how they are transmitting from animals to human or from human to human. People live near to domestic animals because these animals are the main source for their livelihood. They use animals not only for trade, meat and milk but also as a mechanical means for travelling, loading etc. and such close contact increases chances of tick bites. Not only lack of knowledge but due to poverty the rural people are not able to take their ill animals to a very far situated veterinary health service center. People use milk and meat without any knowledge of infection and virus that could be transmitted if the animal is viraemic. (Mallhi TH et al., 2017; Ahmad T et al.,2016). In rural areas farmers and veterinarians are mostly untrained, there is unavailability of vaccines or improper implementation if there is any vaccination program in the area.

The prevalence of CCHF is also steered by geographic characteristics in the regions. We see there is a high level of prevalence in Baluchistan and KPK provinces of Pakistan, boarders of both regions meet to neighbor countries Afghanistan and Iran. The west of Baluchistan is bordered by Iran and its north is bordered by Afghanistan regions. The Khyber Pakhtunkhwa (KPK) and its triable areas (now merged into KPK) situated in the north west of Pakistan boarding with Afghanistan (Khurshid et al., 2015; Atif M et al; 2017).

Both these neighbors are CCHF endemic countries and there is a free transit movement of people and trading of livestock across the borders (Ahmad A et al; 2015).

Seeking healthcare facilities, it is a common practice that people of Afghanistan visit Quetta city hospital, capital city of the of the Baluchistan-province and Peshawar city hospitals, a capital city in Khyber Pakhtunkhwa-province of Pakistan, and Islamabad hospitals. These people from across the border are usually found to be CCHF positive cases, and these are the source of transmitting CCHF infection virus from Afghanistan to Pakistan. In same way there is free movement on border from Iran, people visiting and trading across the border region without any screening system for health. In

Iran, it has been reported that 23 out of 30 provinces are CCHF endemic areas (Chinikar S et al.,2010).

The Baluchistan region holds a large number of livestock animals especially sheep and camel as compared to other provinces of the country. The people always take their animals to other areas; from rural to urban for selling and in this way spreading of the CCHF virus occurs from animals to humans (Atif M et al.,2017).

Baluchistan province is situated in the southwest of Pakistan with holding high numbers of camels, sheep and goats (camels 0.37 million, sheep 12.8 million and goat 11.8 million) (pbs.2020). The area of this largest province covers 44% of the geographical area with 347,190 km^2 of the whole country land. 95% is covered by rangeland while only 5% is arable (Kakar RA et al., 2009 & 2010; Shafiq M et al). The high mortality rate of Baluchistan livestock from infectious disease is due to the non-availability of veterinary services or inadequate supply of medicine for them (Khurshid A et al.,2015). The human fatality rate due to CCHF is up to 20% in Baluchistan region. There is no proper system of surveillance for CCHF data collection in Pakistan. Only incidences-based reports are available (Kasi KK et al., 2019).

Baluchistan region unfortunately faces many problems, one of the most important is poor services of livestock which is very important for healthy and productive husbandry livestock. There is inadequacy of veterinary health services and lack of medicine to the animals in Baluchistan. It is reported that Baluchistan region livestock do not access to the veterinary health services, hardly 11% getting to the available services (Shafiq M et al., 2017). Poor health supply not only reduces the number of livestock animals, but this becomes the cause of the spreading disease such as CCHF among animals and then from animals to the people. The CCHF virus cycle in tick-animals-tick, increase the risk of CCHF virus transmission to human many times. Infected animals do not have visible clinical symptoms for a couple of days up to a week, but they can be identified by serological tests (Leblebicioglu et al., 2015).

There are few numbers of veterinary hospitals, dispensaries or other health services in the country. Usually, veterinary services are available in the urban areas while in the rural areas either they are absent or with improper services (Khurshid A et al.,2015).

The climate and weather of Baluchistan is extreme, hot, arid, little rainfall and cold in winter. The ecology of Baluchistan is agroeconomic, 95% is rangeland while only 5% is arable land. In such environmental condition camels are important and valuable livestock in the Baluchistan province. The camels have high capacity and they can withstand extreme environmental conditions like hot, arid, semi-arid and cold weather. They are adopted for food and water scarcity and can fight with drought and famine condition for the time. Therefore, the camel is a very important and hardworking friend in the life of nomadic people when they move from one place to another place in the search of food and water (Kakar RA et al.,2009 & 2010).

Nomadic lifestyle people are another important factor in the dissemination of CCHF virus from a prevalent area into another new safe area. There are nomadic tribes in the different parts of the country especially they are living on the border between rural and urban areas. To find food and water these people migrate from place to place with season. They move together with camel, sheep, goats and other their animals which are infested with ticks and a real risk for spreading zoonotic disease such as CCHF from endemic areas into the population of new area (Atif M et al., 2017). Camels are an important part of herd mobility in the life of nomadic pastoralists. These nomads use migration between Afghanistan, Iran and Pakistan. They spend their summer time in the upper land of Afghanistan and then in winter migrate to lower land of Pakistan northwest and west region or to Iranian regions on the border (Jasra WA et al.)

We found significant border effect on the areas from neighbor country. Movement of livestock across the border without any health checkup is a serious risk factor of CCHF virus spreading. Often, imported animals are viraemic, then these animals for selling are taken from rural areas into urban and commercials market areas (Karim AM et al., 2017). In rural people, mostly farmer, veterinarian and slaughter men have close contact to livestock but in cities people also come close to these ticks infested animals for trading, particularly in the time of religious festivals when people are buying them for sacrifices. Slaughter men sacrifice such viraemic animals without any precautionary measures due to lack of knowledge of CCHF virus. Many studies have documented that transportation of trading animals from one area to another especially during Eid-ul-Adha festival increase risk of spreading of CCHF virus into new area (Saleem M et al., 2016).

Usually, sacrificial animals are imported to Pakistan from Afghanistan and Iran without having any proper monitoring system (Karim AM et al., 2017). That is the reason why there is highest prevalence of CCHF in bordering regions Baluchistan and KPK.

In both Afghanistan and Iran, the CCHF is endemic with high prevalence of the disease compared to Pakistan. Pakistan shares borders with both these neighbor countries where the movements of people and animals are free, and no system is available for screening which is one of the main causes of spreading and transmitting the CCHF virus into the regions of Pakistan (Ahmad T et al.,2016).

Unfortunately, the Pakistan health infrastructure is not well developed, there is no strategy for coping with any endemic situation. There is also unavailability of trained staff who must be ready in emergency situations for a new disease outbreak (Atif M et al., 2017).

The government needs to make a meaningful strategy to control and prevent CCHF disease in livestock. It is true that there is no proper surveillance system for CCHF and the data is only collected through NIH, working with WHO cooperation. In the prevalence of CCHF, many suspected cases are not diagnosed in the region (Khurshid A et., 2015).

To identify hotspots of CCHF virus, the epidemiology surveillance system has the main role to collect data from different regions and to point out the clusters for the CCHF cases. The information would be useful in the CCHF prevention and control for the future outbreak (Abbas et al., 2015).

The livestock camel, sheep and goat are significant in relation to CCHF incidences. The highest number of camels are present in the Baluchistan. Camels are very useful in the life of these pastoralists and nomadic people. Agrarian use camels for agriculture activity and nomadic people use them as their vehicle for travelling and loading when they are moving from one place to another place. Ali Zohaib et al., (2017) found high seroprevalence of CCHF in camels from Baluchistan and KPK. Other studies reported that camel blood analysis from United Arab Emirate has 67% of CCHF virus seroprevalence and camels in China northwest area with 54% seroprevalence (Camp JV et al., 2020; Li Y et al., 2020).

The goat and sheep have positive significant correlation with the CCHF incidences in the area. The herds of goats and sheep are taken by pastoralist from one area to another for selling. This movements of herds means passing through many streets, villages and cities for trading, particularly in the time of Eid ul Adha festival. This movement of goat and sheep herds might

be the reason that they are significant in the CCHF virus dissemination. We could not find any significant association of other livestock such as cattle and buffalo. This might be because of our dataset, our analysis based on secondhand data for CCHF cases and the livestock data was only available per 2006 census. Another possible reason is that many species in the large ruminants have tick resistance due to a high serum complement level in their blood (Sajid MS et al., 2009; L'Hostis et al., 1996). It would be injustice if we say that large ruminants are not associated with CCHF virus dissemination. Because it is well documented that cattle and buffalos are highly infested with different kind of ticks and they are CCHF viraemic (Farooqi SH., 2017; Zohaib A.,2017).

Developing countries like Pakistan, unfortunately do not have a proper and clear strategy for CCHF infection prevention and control due to lack of coordination between provincial governments of the state. The CCHF is endemic in all the four provinces Baluchistan, Punjab, Sindh and Khyber Pakhtunkhwa of Pakistan and from 5 to more than 100 incidences are reported each year.

In recent years, there is sporadic growing human CCHF cases in the country due to lack of effective surveillance systems (Adnan Khurshid). Establishment of an effective surveillance system is important for the government to collect accurate data in time from all parts of the country. This would not only help in understanding the disease epidemiology and the risk factors, but it will also be useful in making a strategy for control and prevention of CCHF virus transmission (Al-Abri SS et al., 2017; Saleem M et al., 2016).

There is no system available in Pakistan that could be ready for immediate response in the case of a new outbreak. Because there is lack of trained staff that must have knowledge of how to cope with the situation and handle the case. Often, in the case of a contagious infection, professionals keep themselves abstain from caring of patients, not only because of fear but also incompetency (Atif M et al., 2017). Only in big cities, there are a few national laboratories equipped with PCR and other modern technology, which collect samples to diagnose CCHF cases. Otherwise, mostly private laboratories are very expensive, and people do not visit them because everyone could not afford their high cost. Because of these problems there is no proper management and preventive measures. The government needs to make policies at national as well as regional level for CCHF control and prevention. In the case of an outbreak in a region, there is a need to develop isolation and quarantine places to stop the spread of infection into neighbor regions (Yousaf MZ et al., 2018).

Unfortunately, more than half of the population in the country is illiterate, and people do not have knowledge of vectors and pathogens that causes infection. To raise awareness in people about CCHF and tick-borne diseases, health and veterinary education can bring substantial changes in controlling CCHF infection. Media can play a major role to educate the public about CCHF virus and its transmission. Other awareness programs such as workshops, study campaigns or seminars on CCHF must be arranged from time to time throughout the country. These education programs are important, particularly during the period of festivals when there is movement of livestock animals for selling within and across the borders of Pakistan.

9. Limitations & Recommendations

The dataset is based on only NIH laboratory confirmed cases of CCHF, while many confirmed CCHF cases at private laboratory hospital are not recorded to the center of state. Only incidences-based reports are available.

There is a need of active surveillance system that could make possible to collect accurate data and exact number of cases from all regions of the country.

8. Conclusion

There is a high prevalence of CCHF in Baluchistan and Khyber Pakhtunkhwa than Punjab and Sindh regions. In both first two regions of Pakistan, population density is very low, but there the literacy rate is also low compared to Punjab and Sindh regions. Negatively significant association of literacy rate with high level of CCHF cases proves that there is unawareness in public about the disease spreading by ticks.

The border effect which has a positively significant associated with high level of CCHF prevalence in the regions that lie on the border. Unfortunately, both Baluchistan and KPK areas are bordering Afghanistan and Iran where CCHF is endemic. There is free movement of people and transportation of animals across the border that is one main cause of the CCHF diffusion into Pakistan.

Islamabad has a higher literacy rate than all other regions but there is still a high CCHF prevalence. This is not only because of high population density but people from rural areas, particularly from border sites where CCHF is in high prevalence come here for seeking medical facilities in the large city hospitals or to animals selling. For selling, transportation of viremia

animals such as sheep, goats and camels from rural to urban areas is spreading the CCHF virus into new area.

There is a trend of yearly increasing number of CCHF cases, this shows that there is no strategy, or it is not effective and has failed in stopping CCHF spreading in the country. The health care authorities must reconsider the strategy of CCHF control and prevention.

There is an alarming threat for the people of Pakistan if the government is not giving a clear policy to control and prevent the spreading of CCHF. The movement of people and animals from CCHF endemic areas to other safe areas must be controlled through a health screening system. Similarly, to stop the diffusion of CCHF virus from endemic countries, there is a need of a strict monitoring system for the migration of people and transportation of animals across the border. Active surveillance development is indispensable in high CCHF prevalent areas to collect accurate data. The data information would be helpful in controlling the spread of infection and taking effective measurements to prevent CCHF outbreaks.

In the future one could try to find why the other livestock animals such as horses, donkey/mule, cattle and buffalo are not significantly associated with CCHF incidences.

10. Conflict of interest

The author declares that there is no conflict of interest.

11. Acknowledgements

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