



RESEARCH ARTICLE

Seasonal Trends in Seroprevalence of FMD in Bovines under Different Environmental Conditions in Rural KPK, Pakistan

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ABSTRACT

Foot and mouth disease outbreaks and its endemic nature in livestock had always remained a major problem in Pakistan. The present study was aimed to estimate the association between seroprevalence of FMD and ecological variation in selected rural villages of Khyber Pakhtunkhwa province. Meteorological variables from Pakistan meteorological department and sera samples from 2511 cattle and buffaloes were obtained respectively over a period of 1 year “between” July 2013-June 2014. Using 3ABC ELISA serum samples were tested identifying antibodies against FMD serotypes. From a total of 2511 sera, 9.83% tested FMD positive. True prevalence calculated was 7.42%. The highest prevalence was observed in sub humid zone, followed by humid and semi-arid zone. Seasonal pattern of FMD was also identified, showing significantly ($P < 0.05$) higher prevalence in monsoon followed by summer and post monsoon. It was concluded that several factors i.e. farming system, unchecked livestock movements (during religious festivals and trading of livestock from Punjab to KPK) and change in meteorological factors play a vital role in the prevalence of FMD. Based on this pertinent prevention measures are required to reduce future outbreaks considering local climatic conditions.

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INTRODUCTION

Pakistan being an agricultural country is highly dependent on its agriculture. Livestock contributes almost 11.4% in the total national gross domestic product (GDP). Livestock plays an important role in rural economy of Pakistan. 30-35 million populations are directly engaged in livestock rising. Also every 5th Pakistani family is directly or indirectly engaged in livestock farming (Ashfaq *et al.*, 2015; Jamil *et al.*, 2015). Foot and mouth disease (FMD) is an infectious and highly contagious problem of cloven footed mammals, caused by the RNA virus of genus Aphthovirus of family Picornaviridae (OIE, 2012). That exist in seven different serotypes such as, O, A, C, SAT1, SAT2, SAT3 and Asia-1 (El-Moety *et al.*, 2013). FMD is globally distributed, except in Europe, North America, New Zealand and Australia (Kitching *et al.*, 2007). Particularly in Southeast Asia FMD is the vital restraint to dairy productivity and international trade of Cattle and Buffaloes because of enzootic status of FMD in

most countries here (Madin, 2011). Significant scientific literature represents evidence of the impact of climate change on the health and diseases. Significantly focus on vector borne and human health disorders (Rogers *et al.*, 2001). Unfortunately, the impact of climate change on incidence of non-vector borne diseases and animal health has received relatively less attention (Harvell *et al.*, 2002; Mahmood *et al.*, 2016). Considering the global burden of non-vector borne and animal diseases that contributes a lot to poverty in developing countries, attention to these diseases is important.

Climate change, defined as variation in the climate over time, either in average weather or in extreme events (IPCC, 2007). It affects molecular biology of pathogens itself, vectors, land use and farming practices, environmental and zoological factors, and the establishment of new microclimates and microenvironments, therefore influences the distribution, occurrence and prevalence of animal diseases under the varying environmental conditions (Speranza, 2010). Warming of climate has

occurred already in recent decades. If there is any association between climate change and infectious diseases of animals, then there must be dramatic change occurred to certain infections. Standards have been set to associate climate change with infectious diseases (Dion *et al.*, 2012). Many significant animal diseases directly or indirectly are affected by weather and climate. These relations may be spatial, temporal or relate to the intensity of an outbreak.

FMD is endemic in South Asian Countries. Historically FMD has been common in Indo-Pak subcontinent (Zahur *et al.*, 2006) e.g. occurrence in pre-partition of the Punjab, onward 1900 as mentioned in literature (Zulfiqar, 2005). Foot and mouth disease is endemic in Pakistan and its neighboring countries, imposing an extensive negative impact on their livestock industries (Zahur *et al.*, 2006). The spatial distribution of various serotypes isolated and laboratory confirmed in Pakistan (Syed *et al.*, 2013).

The impact of climate factors variation on the transmission and establishment of FMDV in other mammals has been reported from many regions of the world (Ma *et al.*, 2010; Li *et al.*, 2012). Considering published literature and climatic variations over a short distance in Pakistan we hypothesized the impact of change in climatic factors on the prevalence of FMD in bovines in the present study area. Here we represent selection of some associations, which are by no means thorough but are somewhat intended to reveal the assortment of effects. Considering this the present study was conducted to enumerate the FMD seroprevalence and impact of climatic factors on its occurrence.

MATERIALS AND METHODS

Study area: Khyber Pakhtunkhwa (KPK) is located at latitude of 34.95° and longitude of 72.33°. It has three main agro ecological zones based on climate in Pakistan.

- The Semi-arid
- The sub humid
- The humid zone

Classification of study area into agro ecological regions is important in understanding the epidemiology and impact of climate change on FMD prevalence. Livestock farming is the main occupation of rural farming community in Khyber Pakhtunkhwa Province (KPK) of Pakistan. The climate of KPK varies a lot for the size of its nature. Most of the study area average annual daily temperature remains below 23°C but some tropical sub humid regions see higher temperatures of >35°C at some part of the year. Same is the case with precipitation varying all the year in this part of the country. Considering this the present study conducted to find the impact of these climatic factors on seroprevalence of FMD in cattle and buffalo population at different levels and different agro climatic zones.

Meteorological data: The ecological data records were obtained from the meteorological department Peshawar of Pakistan. The data regarding humidity, temperature and annual precipitation was obtained for the study period.

Serum sampling: Serum samples from cattle and buffaloes were collected from all age, both sexes and irrespective of breed from the sampled animals from July 2013-June 2014 in the study area through cross sectional survey. It was conducted to examine seroprevalence of FMD in cattle and buffaloes KPK, Pakistan. The sample size was calculated based on simple random method of sampling techniques, with subsequent predetermined parameters. Previous prevalence in the study area reported (Abubakar *et al.*, 2012), at 95% confidence interval and 1 % specified error.

$$n = 1.96^2 P_{exp} (1 - P_{exp}) / d^2$$

Where n is the required sample size; P_{exp} , the expected prevalence and d is the desired absolute precision. A minimum of 323 samples were likely to be collected in both species at each zone or cluster. A total of 2511 sera samples were tested, using FMD virus nonstructural 3 ABC ELISA, with 97% specificity and 95% sensitivity for identifying of previously infected animals (Engel *et al.*, 2008).

Assessment of FMD incidence through ELISA: In past FMD infected animals could be detected up to 665 dpi using ELISA, antibody levels might start decreasing after six months of the infection (Moonen *et al.*, 2004). Considering the present study most of the animals tested were asymptomatic.

The developed degree of color was directly proportional to amount of the antibody complexes on 96 well plate surfaces and read (at 450 nm) and reference filter was at 492 nm. The final readings for the samples were calculated as follow.

$$\text{Value\%} = \frac{\text{OD sample} - \text{OD negative}}{\text{OD positive} - \text{OD negative}} \times 100$$

If a %OD value was <20% that was considered negative, 20-30% ambiguous and >30% as positive.

Statistical analysis: The ELISA test results for tested animals, its demography (sex, age, specie) and season of infection with climatic zones were recorded. Chi square test was used to find the association between categorical variables and Descriptive statistical analysis was carried out using SPSS version 20.0 was used.

RESULTS

Table 2 represents the chi square analysis of the data. Statistical analysis of the data showed an overall prevalence of 9.83% in cattle and buffalo population of rural KPK. While it was higher in buffalo (13.57%) significantly ($P < 0.05$) than cattle (6.02%) (Table 2). The results show that FMD is endemic in all regions of rural KPK. Age (young <1 year of age, adult >1 year) analysis had no significant ($P > 0.05$) impact on the prevalence of FMD both in cattle and buffaloes. Based on agro-ecological classification the results showed a statistically different prevalence rate of FMD in the present study area divided into three distinct zones. The highest prevalence

of FMD was calculated in Sub-humid zone followed by humid and semi-arid zone.

Sero-prevalence of FMD: In the present study total of 2511 sera samples were screened for FMD through 3ABC ELISA to calculate the sero-prevalence of FMD in the present study area. The ELISA results were interpreted as per manufacturer's recommendations. Total of the final 3ABC ELISA results are given in figure 1. The results showed that 186 sera samples from cattle and buffaloes out of 2511 were having corrected optical densities percent values of greater than 30. 481 samples scored non-detectable values OD values. While 1844 samples scored a negative OD values less than 20.

Effect of Climatic factors on prevalence of FMD: Data regarding climatic factors i.e. weekly average temperature, humidity and rainfall was analyzed with respect to FMD sero-prevalence in samples collected in the same week to check the impact of these factors on FMD occurrence. Observed temperature and humidity fluctuation was most pronounced at the higher altitudes. The results showed that increase in temperature with increase in humidity was significantly associated with the incidence of FMD as depicted in table-1 and 2. With increase in rainfall in the study area more cases of FMD were detected. After thorough analysis of questionnaire based data it was concluded that increase in number of cases with respect to increase in temperature and humidity was due to high interaction of animals at grazing and water spots with ascending animal free movements. In summary the association of climatic factors variation is demonstrated here playing a vital role in the spread and transmission of FMD in the study area.

Secular trends in FMD prevalence: Temporal indicators i.e. time and season of FMD was studied. Data regarding FMD case with respect to date and season in which it was sampled was analyzed. A clear trend was observed for FMD in association with climate variations. A higher prevalence (40.08%) of FMD was calculated in monsoon (July-Sep) season (Table 2). Figure 2 shows the monthly incidence of FMD in the study area. The months in higher prevalence of FMD was found having elevated average weekly temperature and high humidity. For temporal distribution data of samples collected for one year was analyzed. A trend of increasing incidence of FMD could be seen in hotter months of the year and gradual decrease in cooler months.

DISCUSSION

Foot and mouth disease is enzootic in all most all parts of Pakistan. Pakistan having a large livestock population faces lots of uncontrolled infectious and non-infectious health problems. FMD is one of the most important infectious diseases, hindering the production of livestock and causing large economic losses in this sector. FMD remains mostly uncontrolled in Pakistan, as in general prophylactic vaccination is not practiced, being practiced by small portion of farmers. In a country where

FMD is enzootic like Pakistan, and where large population of susceptible livestock exist, with imperfect vaccination, disease reporting and investigation, such serological surveys are very important in understanding the epidemiology of FMD. To build up a prompt epidemiological profile of the enzootic FMD occurrence in KPK, 2511 sera samples of cattle and buffalo from various existing agro ecological zones were investigated, using 3 ABC ELISA, and a seroprevalence of 9.83% was obtained. This figure (9.83%) is significantly ($P < 0.05$) lower from the previous prevalence reported (Abubakar *et al.*, 2012; Syed *et al.*, 2013). The difference in the results may be due to the difference of study area within the same country. Where differences in management practices, herd size and movement of animals exists, as also attributed by (Syed *et al.*, 2013) The herd size in this part of the country is too small usually as compared to the other provinces because of limited agricultural land to support the animals. The low prevalence than the previous studies may also be due the geography of the study area, as previously low prevalence reported by (Abubakar *et al.*, 2012) in hilly areas. Relation of FMDV persistence with increase in humidity and temperature was also found i.e. that in monsoon and summer season where the temperature gets hot and humid in the study area also the occurrence of FMD cases increases with it. This relationship was found in agreement with those results already reported (Dion *et al.*, 2012). The elevated incidence of FMD cases in hot humid regions is due to the suitable environmental conditions for persistence and multiplication of FMDV as previously reported by (Hii *et al.*, 2011). The persistence of air borne viruses supported by elevated humidity (Minhaz *et al.*, 2010)

The climate impact on infectious disease prevalence in the same study area was also reported (Khan *et al.*, 2015). The monthly different incidence pattern of FMD in present study may be due to the movements of animals higher in hotter months as compared to colder months that play a major role in the transmission of FMD. In hotter months farmers takes their animals for grazing and same water source are mostly used in this area by animals brought from different regions as previously reported in South Africa (Dion *et al.*, 2011). The seroprevalence of FMD in buffalo population was significantly higher as compared to cattle. It may be because of the free movements of buffaloes being brought all the year from Punjab to this part of the country, as previous studies have shown higher prevalence of FMD in Punjab as compared to other parts of the country. Also buffaloes have to face environmental stress of higher precipitation level at higher altitudes as compared to cattle. The cattle population in KPK is mostly local or exotic breeds that are habitual to such climatic conditions that's why they were less affected by infectious and non-infectious diseases in this part of the country as is evident from the previous studies (Khan *et al.*, 2015). The results of the present study will be useful in future for launching control programs in the country, knowing the high FMDV prevalent zones and trends and pattern of FMD in time and space.

Conclusions: Change in climate i.e. increase in temperature and humidity was found having direct impact

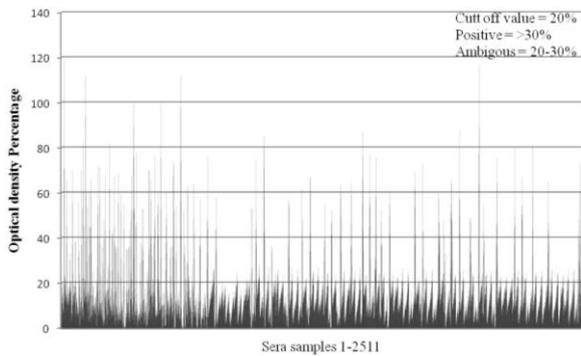


Fig. 1: Optical density percentage values distribution for serum samples of 2511 investigated with FMD-3ABC ELISA for year 2013-2014 in Khyber Pakhtunkhwa.

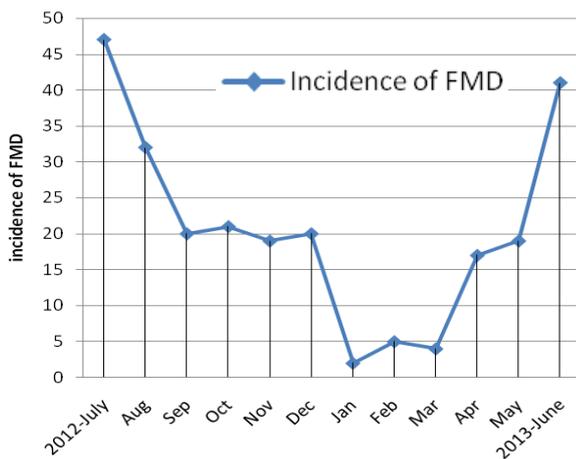


Fig. 2: Pattern of temporal distribution of FMD in KPK for the year 2012-2013 in bovines.

Table 1: Comparison of Seroprevalence of FMD in different agro ecological zones of KPK, Pakistan in 2012-2013

Agro ecological zones	No. of Sera samples tested	No. of samples tested positive	Calculated prevalence (95% C.I)	True prevalence
Semi-Arid zone	1389	94	6.71 (3.17-11.45)	3.26%
Sub-Humid zone	615	96	15.60 (4.80-61.21)	13.69%
Humid zone	507	57	11.24 (4.27-35.52)	8.95%
Total	2511	247	9.83%	7.42%

True Prevalence (TP) = $AP+ (Sp-1)/Sp-(1-Se)$ [28]. Where AP=apparent prevalence, Se=Sensitivity, Sp=Specificity (95% CI); We have given Test sensitivity=95% and Specificity 97%.

Table 2: Description and prevalence of FMD in cattle and buffaloes in KPK, 2012-2013

Description	Variables	Level	FMD sero-prevalence (%)	P-value
Host specie	Cattle/ Buffaloes	Cattle	6.02(76/1251)	0.0006
		Buffaloes	13.57(171/1260)	
Age*	Young/Adult	Young	10.02(111/1107)	0.0787
		Adult	9.67(136/1404)	
Season	Winter/ Summer/ Monsoon/ Post Monsoon	(Dec-March)	5.66(14/247)	0.00009
		(Apr-June)	31.17(77/247)	
		(July-Sep)	40.08(99/247)	
		(Oct-Mid Dec)	23.30(57/247)	

*Non-significant impact

on the prevalence of FMD in bovines. Proper prophylactic vaccination can control FMD occurrence if the free moments of animals could be restricted. Also the awareness of farmers on the importance of vaccination and disease reporting is crucial in control of FMD.

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Author's contribution: AK designed research, collected samples, data analysis and manuscript write up. MHS and MUDA developed and designed over all idea and actively remained involved in laboratory analysis and manuscript preparation. ZF and AK contributed in sample collection and manuscript write up.

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