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Brief Report

Spatial Trend of Foot and Mouth Disease Virus (FMDV) Serotypes in Cattle and Buffaloes, Pakistan*

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The present study describes the frequency of Foot and Mouth Disease (FMD) virus serotypes (O, A and Asia-1) in major regions (all provinces) of Pakistan using Indirect Sandwich ELISA. Also, spatial distribution of various FMD serotypes and their comparison is discussed. A total of 590 samples (Epithelial tissue) have been analyzed during a period of five years (2005–2009). Out of 590 samples, 180 were found positive, giving an overall confirmation of FMDV about 33.2 %. Of the prevalent serotypes, FMDV 'O' serotype caused most outbreaks (20.7 %), followed by serotype A (6.6 %) and serotype Asia-1 (4.6 %) while there was no positive case of type 'C'. The study clearly showed that the disease was more frequent in the agro-climatic zones than in hilly areas. Based on the data of 590 samples (>50 outbreaks), the overall prevalence of FMDV in cattle and buffaloes in Pakistan was 33.2 %, while in cattle alone, it was 37.1 %, higher than in buffalo (28.7 %). There were eight cases of mixed serotypes infection, indicating the presence of endemic state of disease. Another significant feature was the change over time. In phase-I (2005–2007), there was an overall prevalence of 29.4 %, while the occurrence of the serotype O, A and Asia-1 was 20.4 %, 2.9 % and 4.7 %, respectively. During phase-II (2008–2009), the overall prevalence was 59.21 %, while those of serotype O, A and Asia-1 were 22.4 %, 31.6 % and 4.0 %, respectively. This clearly indicated a shift from serotype O to A, which may help to explain the occurrence of more severe outbreaks, despite vaccination.

Spatial and Temporal Distribution; Serotype shift; Foot and Mouth disease (FMD) Virus; Indirect Sandwich ELISA; Serotypes

Foot-and-mouth disease (FMD), a highly contagious viral disease affecting primarily cloven-hoofed animals, continues to be a major concern for the world livestock industry. At one time or another, this menace occurs in most parts of the world including Pakistan, often causing epidemics in cattle, buffaloes and swine. Sheep, goats and many species of wildlife are also susceptible. FMD mortality is usually low but its morbidity is high. Mortality in young calves may be as high as 70 % but in adults it is seldom higher than 5 %. The virus mainly enters the body either by inhalation (droplets) or ingestion (contaminated food). There are seven distinct types of FMD virus viz A, O, C, Sat-1, Sat-2, Sat-3 and Asia-1. The virus is highly mutating leading to constant emergence of new subtypes. The virus is found in blood, milk and saliva soon after infection. Animals of wild origin may act as carrier or reservoir, e.g. dogs, cats, deer, wild boars and even humans, birds and flies[10].

It is important to know the prevalence of major transboundary animal diseases like foot and mouth disease so that a national policy can be determined to control these diseases. A review by Kesy[5], concludes FMD is currently present worldwide. In 2000, fifty nine countries officially reported outbreaks of FMD but there is very little data available from Pakistan. Also the data which was available is based on complement fixation test (CFT) which is non- specific[2] so the data given in this study is much more reliable and authentic and also covers a wider range of areas all over the country.

If we consider the occurrence of FMD in Pakistan, according to Perez *et al.*[9] method, the estimated probability of an FMD outbreak per 25 km^2 cell would be ranged from 0.017 to 0.812, with a range of 47.8 fold (0.812/0.017). The Punjab region would be most likely to have an outbreak.

In view of the importance of FMD, the data of five years (2005–2009) has been gathered and analysed to establish the occurrence of FMDV serotypes in different areas of the country.

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MATERIALS AND METHODS

Study area and sample collection

Samples were collected throughout the country and submitted by the District Diagnostic Laboratories, following the procedures described by the Office International des Epizooties[8]. The disease was suspected in cattle and buffalo having mucosal and/or foot lesions. The preferred tissue for diagnosis was epithelium from un-ruptured or freshly ruptured vesicles. Samples were taken aseptically in buffered glycerine and dispatched to the laboratory at refrigeration temperature. Before freezing (-20 °C), the containers were carefully sealed using screw caps.

Laboratory diagnosis using Indirect Sandwich Enzyme Linked Immunosorbent Assay (Is-ELISA)

The ELISA kits used for the detection and confirmation of FMD serotypes were supplied by BDSL, Pirbright, UK. This is an indirect ELISA test for FMDV antigen detection and serotyping[3] in which different rows in multi-well plates were coated with rabbit antisera to each of the four serotypes of FMD virus (Serotype, O, A, C and Asia-1). A color reaction on the addition of enzyme substrate indicated a positive reaction. With strong positive reactions this was evident to the naked eye, but results were read spectrophotometrically (Immunoskan, BDSL, Finland) at an appropriate wavelength (492 nm). In this case, an absorbance reading greater than 0.1 above background indicates a positive reaction for FMDV.

RESULTS

A total of 590 epithelial samples collected over a period of five years were analyzed for the presence of FMD (2005–2009). Out of these samples, 180 were found positive for FMDV, giving an overall occurrence of about 33.2 %. Of the prevalent serotypes, 'O' type FMDV accounted for the most outbreaks (20.7 %), followed by A virus type (6.6 %) and Asia-1 virus type (4.6 %) while there was no positive case for type 'C' (Table 1 and 2). The study showed clearly that frequency of the disease was more in the agro-climatic zones and less in hilly areas (Fig. 1).

Based on the data of 590 samples (>50 outbreaks), the overall prevalence of FMDV in cattle and buffaloes in Pakistan was 33.2 % while it was higher in cattle (37.1 %) than buffalo (28.7 %) (Table 3). There were eight cases of mixed serotype infection. There was an indication of serotype shift when we divided the data into two phases. In phase-I (2005–2007), there was an overall prevalence of 29.4 %, while the occurrences of the serotypes O, A and Asia-1 were 20.4 %, 2.9 % and 4.7 %, respectively (Table 1). During the phase-II (2008–2009), there was an overall prevalence of 59.2 %, while the occurrence of the serotypes O, A and Asia-1 were 22.4 %, 31.6 % and 4.0 %, respectively (Table 2).

DISCUSSION

There were two major purposes for the above study, the first was to implement a reliable diagnostic facility for FMD diagnosis and the second was to report the major serotypes of FMDV present in different animal populations. So the study considered the spatial distribution of different FMD serotypes and their frequency over a period of time.

The study showed that the overall prevalence of FMD is about 33.2 % while serotype 'O' FMD virus accounted for most of the outbreaks (20.7 %), followed by the 'A' (6.6 %) and Asia-1

Table 1. Province-wise FMD serotypes confirmed from outbreaks of 2005–2007

Area with Type of Zone	Total samples (514)	Positive samples (151)	Different serotypes in positive samples			
			O	A	Asia 1	Mixed
Punjab (Agro-Climate)	(222)	60	40	02	13	05
Sindh (Agro-Climate)	(84)	26	20	01	05	--
Khyber Pakhtunkhwa (Mixed region)	(135)	31	22	03	05	01
Balochistan (Mixed region)	(28)	14	04	09	01	--
Azad Jammu and Kashmir (Hilly)	(14)	04	04	--	--	--
Gilgat Bultistan (Hilly)	(31)	16	15	--	--	01
Total	= 514	151	105	15	24	07
	Percent Prevalence	29.4	20.4	2.9	4.7	1.4

Table 2. Area-wise FMD serotypes confirmed from outbreaks of 2008–2009

Sr. #	Area with Type of Zone	Total Samples (76)	Samples positive (45)	O	A	Asia 1	Mixed
1	Punjab (Agro-climate)	53	32	14	17	--	01
2	Sindh (Agro-climate)	18	10	01	06	03	--
3	Khyber Pakhtunkhwa (Mixed region)	05	03	02	01	--	--
	Total	76	45	17	24	03	01
	Percent Prevalence		59.21	22.4	31.6	4.0	1.3

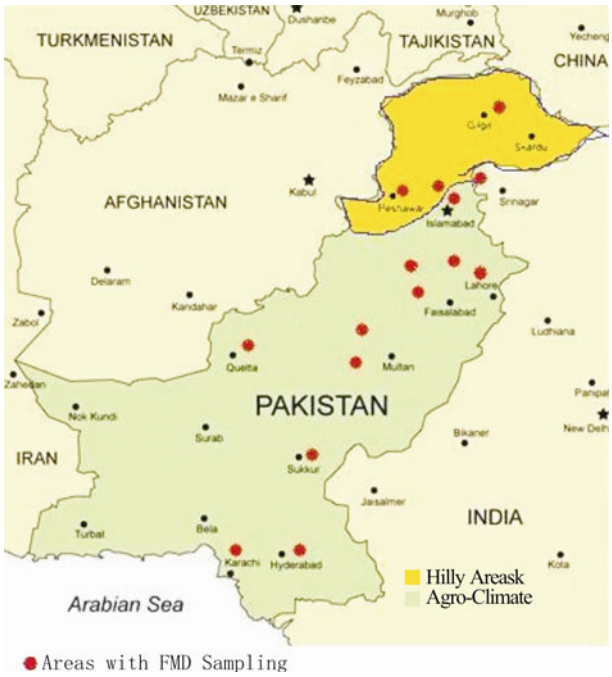

Fig. 1. Comparison of FMD outbreaks in hilly and agro-climate areas. Hilly, darker grey; Agro-Climate, lighter grey.

virus types (4.6 %). There is a 3 to 1 difference between the number of samples collected and the positives. The possible explanation for this would be that the sample collection, preservation and dispatch to laboratory need more attention. In this regard, more training of field staff on sample collection is recommended. The sample quality was much improved in the second period (2008–2009) as the launch of different projects on FMD focused on increasing the capability of veterinary field staff in outbreak handling and sample collection.

Serotype 'O' is the most prevalent serotype in our study, which is in accordance with Knowles *et al*.[7] who described a particular genetic lineage of this serotype, which they named the PanAsia strain, and which was responsible for an explosive pandemic in Asia extending to parts of Africa and Europe from 1998 to 2001. In another recent report from the region, Rashtibaf *et al*.[11] estimated the frequency of FMD virus (FMDV) in cattle slaughtered in Mashhad abattoir, Mashhad, Khorasan Razavi, north-east of Iran. The results showed that 37.7 % of cattle (96 of 255) were carriers of the virus. Among 96 positive samples, 58 (60.4 %) belonged to serotype O. Nucleotide sequencing and phylogenetic analysis showed close similarity between viruses lineage and isolate region in Pakistan. The high incidence of serotype O from our study also indicated that it is the commonest in the region.

Jamal *et al*.[4] also reported the same distribution of different serotypes of FMDV in Pakistan during the period 1952–2007. During this time, serotype O was found to be the most prevalent ($p<0.001$) followed by serotype Asia-1 and A, which was slightly different from our findings as we split the data. During the second phase of above study (2008–2009), we reported

Table 3. Specie-wise prevalence of FMD virus

Species	Positive	Total	Percent Prevalence(%)
Cattle	117	315	37.1
Buffalo	79	275	28.7
	196	590	33.2

an overall prevalence of 59.2 %, while the occurrence of the serotype O, A and Asia-1 was 22.4 %, 31.6 % and 4.0 %, respectively.

In a similar study from India, Bhattacharya *et al*.[1] reported the prevalence of the four FMD serotypes: O type virus (67 %), Asia-1 (15 %) A (14 %) and C FMD (4 %), and no cases have been recorded since 1996. Our results are also in close agreement with Klein *et al*.[6] who conducted FMD studies in the Landhi Dairy Colony, Karachi, Pakistan (the largest buffalo colony in the world). According to their findings, 19 samples belong to the regional PanAsia II lineage of serotype O while 56 belong to the A/Iran/2005 lineage of serotype A. Similarly, we have also reported the higher incidence of serotype A during the period 2008–2009.

Another important finding is the occurrence/confirmation of more than one serotype of FMD from same outbreak. A possible explanation in some cases would be the cross contamination of samples in the field as the same person can be involved in outbreak attendance at more than one location. It is also possible that due to unrestricted animal movement and endemic nature of FMDV, more than one serotype was circulating in the same area.

The study also showed that incidences of the disease were highest during the winter and early spring months and in the agro-climatic zones and less in hilly areas. Due to the unrestricted movements of animals among different cattle markets, the disease was transmitted either by direct contact or by aerosols from infected to healthy animals; these findings are in congruent with Jamal *et al*.[4] who attributed this to the livestock movement in the country particularly due to religious festivals.

The above data is important to realize the serotypes of FMD prevalence in the cattle and buffalo population and to make the proper vaccination strategy for FMD progressive control program in the country.

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