

## INVESTIGATIONS OF FOOT AND MOUTH DISEASE OUTBREAKS IN DIFFERENT DISTRICTS OF PUNJAB, PAKISTAN

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### ABSTRACT

Foot and mouth disease (FMD) is an endemic and highly contagious disease of ruminants and its outbreaks had always remained a threatening problem in Pakistan. The aim of the present study was to determine the prevalence of FMD and its serotypes in ruminants of different districts of Punjab. Sampling was performed from outbreaks of foot and mouth disease in different districts of Punjab, Pakistan including Khanewal, Faisalabad and Chakwal. An indirect sandwich Enzyme Linked Immunosorbent Assay (IS-ELISA) was employed for the detection and typing of Foot and mouth disease virus. A total of 19 outbreaks in cattle, buffaloes, sheep and goats suspected of FMD were attended, investigated and 109 epithelial tissue samples (42 from buffaloes, 54 from cattle, 10 from goats and 3 from sheep) were collected. A total of 77 (70.65%) samples were found positive for FMD virus. Out of these positive samples, 48 were successfully typed into serotype O (62.33%) followed by 26 (33.77%) into Asia I and 3(3.90%) into serotype A. FMD was more prominent in Faisalabad as compared to Khanewal and Chakwal. The disease and all its three

serotypes were predominant in cattle (85.18%) as compared to buffaloes (64.28%) and goats (40%). The results of the present study showed that FMD is very common disease in large as well as small ruminants of Pakistan and a proper vaccination program should be planned and implemented for its control to avoid the losses caused by this devastating disease.

**Keywords:** *Bubalus bubalis*, buffaloes, foot and mouth disease, ruminants, serotypes, IS ELISA, Punjab

### INTRODUCTION

Foot and mouth disease is an acute and infectious disease of domestic animals including cattle, buffalo, sheep, goats and swine having communicable potential (Longjam *et al.*, 2011). FMD virus has positive polarity and it is a single stranded virus having RNA as a genomic material. It belongs to Family *Picornaviridae* and genus *Aphthovirus* with seven different serotypes O, A, C, Asia1, SAT 1, SAT 2 and SAT 3, which are antigenically and immunologically different from

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each other. Each serotype has a vast range of antigenically different subtypes (Kitching *et al.*, 2005). This disease is characterized by high fever, formation of fluid filled vesicles in mouth, buccal mucosa, nares, feet and teats (Callens and De Clercq, 1997). FMD does not cause high motility in adult animals but drastically reduce the milk production and affects the reproductive ability of animals. Infected animals usually shed virus before and after the onset of clinical signs. In young animals mortality is mostly due to myocardial degeneration also known as Tiger Heart Disease (Nawaz *et al.*, 2014).

The difficulty regarding the control of foot and mouth disease is due to its wide host range and geographical distribution along with poor cross immunity, antigenic diversity and establishment of a carrier state (Mdetele *et al.*, 2014). Small ruminants play an important role as reservoirs for FMD outbreaks in cattle and buffaloes (Gelaye *et al.*, 2009). Mostly small ruminants like sheep and goats exhibit silent signs of disease, which are usually mislooked by the farmers (Mishra and Ghei, 1983). The cumulative prevalence of FMD virus in cattle and buffaloes in Pakistan was 33.2%, separately in cattle it was 37.1% which is higher in comparison with buffaloes (28.7%) and other ruminants (Abubakar *et al.*, 2012).

In Pakistan commonly used diagnostic techniques for FMD are combination of virus isolation and ELISA and in some advanced laboratories reverse transcriptase Polymerase Chain Reaction is used (Reid *et al.*, 1999). The ELISA procedure is used in many institutes and laboratories for detection of FMD antigen but still the margin of improvement in antigenic sensitivity is present. Virus isolation in combination with ELISA techniques produces far much better results as compared to direct ELISA (Reid *et al.*, 1998).

The estimate of prevalence is a primary requisite before going to implement any preventive and control measures. So the present study was conducted to know the prevalence of foot and mouth disease in cattle, buffaloes, sheep and goats in geographically different districts of Punjab, Pakistan.

## MATERIALS AND METHODS

### Study area

The current study was carried out in geographically different districts of Punjab, Pakistan including Khanewal (30.18°N, 71.56°E), Faisalabad (31.25°N, 73.44°E) and Chakwal (32.55°N, 72.51°E) representing lower, central and upper portion of Punjab (Figure 1). The selected districts are diverse regarding their livestock population, climatic conditions, average rainfall (approx. 130 mm, 300 mm and 500 mm) and height above sea level (approx. 430 feet, 605 feet and 1637 feet) respectively.

### Sample collection

Based on typical clinical signs of FMD, 109 samples of diverse type were collected from 19 different outbreaks reported from different towns and tehsils of study districts. The samples were collected from July 2013 to July 2015. A written consent was collected from the owner of all animals who participated in the study. The specific techniques as per standard international safety rules and ethics were adopted during the whole span of research. The mouth swab samples were collected in sterilized 15 ml plastic falcon tubes having 8 to 10 ml glycerolized buffer saline with pH 7.2 to 7.6. The sampling procedure was done with the help of field veterinary officers and assistants. Collection

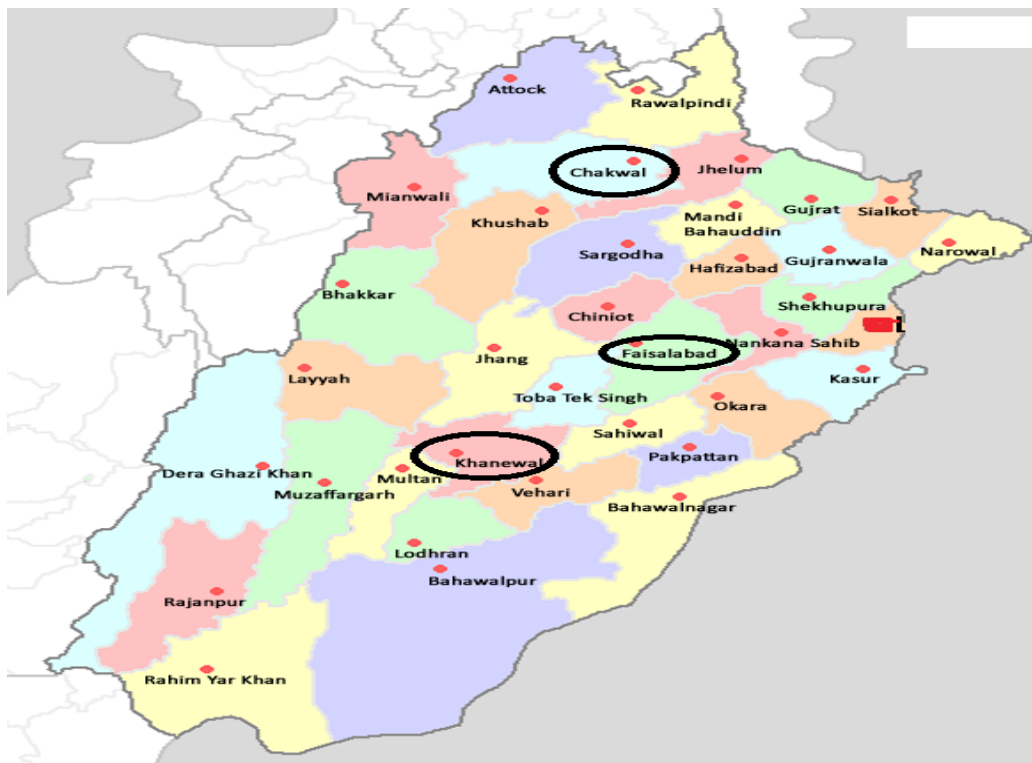


Figure 1. Map representing geographical location of study districts of Punjab, Pakistan.

of samples and addition of antibiotics to avoid secondary bacterial growth was done according to the guidelines of Office International des Epizooties (OIE, 2008). The samples were stored at  $-80^{\circ}\text{C}$  till further processing.

#### **Detection of FMD serotypes using indirect sandwich ELISA (IS-ELISA)**

To detect the FMD virus and its serotypes (A, O, Asia-1 and C) in buffaloes, cattle, sheep and goats an indirect Sandwich ELISA kit made by Institute for Animal Health, Pirbright Laboratory, UK was used. Briefly the wells of 96 well plates were coated with rabbit antiviral serum to each of the three serotypes of FMD virus (Serotype A, O and Asia-1). Incubation and washing was performed before and after adding of suspected

sample. After that Guinea pig anti serum was added followed by Rabbit anti guinea pig immunoglobulin conjugated to Horseradish Peroxidase (HRP). A positive reaction is indicated by the production of specific color after addition of substrate. Strongly positive reactions can be seen by naked eye, but results were read by ELISA reader (Biotek, USA) at wavelength 492 nm (Ferris and Dawson, 1998).

#### **Statistical analysis**

The data obtained was analyzed by using Chi square test with the help of Minitab Software version 15.0 (Minitab Inc, State College, PA, USA).

## RESULTS AND DISCUSSION

Foot and mouth disease is the most important viral disease of ruminants with the potential to cause heavy economic losses. These losses includes deaths in young ones, marked reduction in milk production, abortion at advanced level of pregnancy, reduced working ability and quality of semen. The results of the conducted study showed that the cumulative prevalence of FMD virus in different districts of Punjab, Pakistan was 70.65% which was close to the results of Habib *et al.* (2014) who reported 78% occurrence of FMD virus in Faisalabad. The recorded prevalence is much elevated to the previous findings of (Abubakar *et al.*, 2009; Abubakar *et al.*, 2012) which showed an increasing trend in FMD prevalence.

Among the different geographical locations FMD was found non significantly ( $P > 0.05$ ) highest in Faisalabad 79.35% and lowest in Chakwal 41.65% which was in agreement with the findings of Anjum *et al.* (2006) who reported lowest FMD prevalence in Chakwal (Table 1). The results indicate that the disease was prevalent in all districts but higher percentage in Faisalabad and Khanewal was may be due to large number of dairy sheds and congested population of livestock in these districts (Nawaz *et al.*, 2014).

The present research revealed that among the different serotypes prevailing in Punjab province, serotype “O” statistically ( $P < 0.05$ ) appeared to be the most prevalent (62.33%) followed by type “Asia-1” (33.77%) and “A” (3.90%). Serotype O and Asia I were most frequent in Faisalabad while Serotype A was prevalent in Khanewal. Serotype Asia I is the only reported type from district Chakwal as shown in (Table 1). Similarly Jamal *et al.* (2010) reported the distribution as serotype O most prevalent ( $P < 0.001$ ) followed by type

Asia-1 and A. The same pattern of results was also found in large ruminants of Pakistan (Abubakar *et al.*, 2012; Habib *et al.*, 2014). In neighboring countries (Bhattacharya *et al.*, 2005) reported the prevalence of three serotypes, and found “O” type involved in most outbreaks (67%), followed by “Asia-1” (15%) and “A” (14%) in India. Serotype ‘O’ was the most involved type reflects the results of Knowles *et al.* (2005) who demonstrate that a special genetic lineage of this serotype (PanAsia strain) was accountable for a blasting pandemic in Asia which further extends to various locations of Africa and Europe up to 2001.

The prevalence of FMD was highest in cattle (85.18%) than in buffaloes (64.28%) and goats (40%), while no sample from sheep showed positive results. There was a significant variation in the prevalence of FMD in all the species (Table 2). These finding were supported by the observation of Abubakar *et al.* (2012). Regarding small ruminants, prevalence of FMD in this study was higher in goats (40%) as compared to the results of Lazarus *et al.* (2012) who found (21.81%).

Individually each serotype was also more prevalent in cattle population as compared to other animals. It may be due to introduction of extensive exotic cattle blood and cross breeding which leads to high susceptibility of cattle towards FMD (Zulfiqar, 2003).

The results of the conducted study showed that the foot and mouth disease virus was more predominant in female animals with 72.86% (51/70) prevalence in comparison with males who showed 66.66% (26/39). Though the percentage of FMDV is higher in females than males but the difference between these genders was statistically non significant ( $P > 0.05$ ) as shown in Table 3. All the present serotypes were also more common in female population individually which was

consistent to some previous findings (Awan *et al.*, 2009). Females of large ruminants and small ruminants are more prone to FMD is also evident from the recent results from Punjab, Pakistan (Nawaz *et al.*, 2014; UrRehman *et al.*, 2014). The greater percentage of FMD virus and its serotypes in females might be due to certain stress condition which includes oestrus and pregnancy. Lactation

and nutritional deficiencies also affect their resistance to infection (Susan and Mays, 1998).

It is concluded from above data that FMD is an endemic problem of Pakistan preferably with serotype 'O'. It is recommended that intensive vaccination programme should be adopted in large ruminants to control the adverse effects of disease and its economic losses to farmers as well as small

Table 1. Geographical and serotype based prevalence of FMD in Punjab, Pakistan.

District	Total samples	Positive samples	Different serotypes in positive samples		
			Serotype 'O'	Serotype 'Asia1'	Serotype 'A'
Khanewal	34	22 (64.70%)	14	06	02
Faisalabad	63	50 (79.35%)	34	15	01
Chakwal	12	05 (41.65%)	--	05	--
Total	109	77 (70.65%)	48 (62.33%)	26 (33.77%)	03 (03.90%)
Non significant (P>0.05)			Significant (P<0.05)		

Table 2. Species based prevalence of FMD in Punjab, Pakistan.

Location	Cattle	Buffalo	Goat	Sheep
	Positive/ sampled	Positive/ sampled	Positive/ sampled	Positive/ sampled
Khanewal	09/12	09/14	04/06	00/02
Faisalabad	34/38	16/20	00/04	00/01
Chakwal	03/04	02/08	--	--
Total	46/54 (85.18%)	27/42 (64.28%)	04/10 (40.00%)	00/03 (00.00%)

Table 3. Sex based prevalence of FMD in Punjab, Pakistan.

Location	Male		Female	
	Total sampled	Total positive (%)	Total sampled	Total positive (%)
Khanewal	12	06	22	16
Faisalabad	21	16	42	34
Chakwal	06	04	06	01
Total	39	26 (66.67%)	70	51 (72.86%)

ruminants who act as carrier to silently spread FMD.

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