

EPIDEMIOLOGICAL INVESTIGATION OF FOOT AND MOUTH DISEASE IN BOVINES OF FAISALABAD

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ABSTRACT

A cross sectional analytical sero epidemiological investigation was carried out in district Faisalabad during 2012 with the objective to determine the seroprevalence of foot and mouth disease (FMD) and identifying the risk factors associated with the disease in bovines. Total 352 serum samples were collected from buffaloes (n= 212) and cattle (n= 140) from eight towns of Faisalabad and examined at Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan using non structural proteins (NSP) enzyme linked immunosorbent assay (ELISA). Results showed that the overall seroprevalence of FMD in Faisalabad was (28.40%). Higher seroprevalence was observed in cattle (40%) followed by buffaloes (20.75%). Significantly higher seroprevalence (38.63%) was recorded in Tandlianwala town and lowest in Jinnah town (18.18%). From the various risk factors like age, species and herd type were found to be statistically associated with the disease ($p<0.05$). Whereas, no significant variation was observed in relation to sex and pregnancy. It was concluded from the results that the seroprevalence of FMD in bovine population is of concern in the area. Therefore, effective control measures should be adopted to limit the effect of disease.

KEYWORDS: Epidemiology; seroprevalence; food & mouth diseases; risk factors; bovines; Pakistan.

INTRODUCTION

Foot and mouth disease (FMD), is a highly contagious disease caused by virus belonging to the genus *Aphovirus* of family *Picornaviridae*. The genome is slightly more than 8 kb in size and code for four different structural proteins (VP1, VP2, VP3 and VP4) that arrange to make an icosahedral capsid and some non structural proteins (NSPs) (15). There are seven serotypes of FMD virus i.e. A, Asia-1, C, O, SAT-I, SAT-II, and SAT-III. In Pakistan, the most common serotypes are O (70%), Asia-I (25%) and A (4.67%) causing a loss of more than Rs. 6.00 billions to farmers every year (20). FMD is characterized by fever, lameness, salivation, and vesicular

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lesions on tongue, feet and teats (12) and cause myocardial degeneration in young animals, known as tiger heart disease which causes mortality (10).

Overall prevalence of FMDV in bovines of Pakistan was 33.2 %, while in cattle it was higher (37.1%) than buffalo (28.7%) which is an indication that virus is endemic in the country (2). Its frequent epidemics cause heavy economic loss to cattle and buffaloes. The livestock population of Pakistan is gravely susceptible to FMD with remarkable variation in the severity, course and prevalence of disease due to the fact of cross breeding of local with exotic animals and import of exotic breeds (11). Punjab is at high risk due to its geographical location as compared to other provinces of Pakistan as there were 758 outbreaks of foot and mouth disease in Punjab reported during the years 2002 to 2005 (3). FMD in buffalo and cattle ranked 1st with reference to morbidity rate of 10.3% and 9.9% respectively in Punjab (4). Serology of FMD is particularly important because it can detect antibodies in a range of livestock infected as well as in animals with mild infection (5). The recent developments of ELISA tests against 3ABC (NSP) have greatly enhanced the sero surveillance of FMD as they detected exposure to live virus for all the seven serotypes even in vaccinated herds (6).

Since there is no recent data on epidemiology of FMD in Faisalabad, there is a need for regular and continuous surveillance of the disease and identification of its associated risk factors to protect our precious animals and to reduce the annual economic loss of farmers. Present study was conducted to determine the seroprevalence of FMD and identification of certain risk factors associated with the disease.

MATERIALS AND METHODS

Study area

The study was carried out in District Faisalabad located in northeast part of Punjab province between longitude 73°74 East and latitude 30°31.5 North. The average annual rainfall and temperature range between 250-300 mm and -2° to 50°C respectively.

Study design

A cross sectional epidemiological study was conducted and a questionnaire was designed to collect information from the animal owners on selected herds. Risk factors such as age, sex, species, herd type and pregnancy

status were analysed. On the basis of age, animals were divided into three groups (<2 years, 2-4 years and >4 years). Blood samples (10 ml) were aseptically collected from jugular vein of randomly selected buffaloes and cattle of district Faisalabad using vacutainer tubes and an identification code was marked at each sample. The sera were separated and shifted to serum tubes and transported to Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan using an ice box. Sera were heat inactivated at 56°C for 30 minutes and then kept at -20°C till further processing.

The serum samples were analysed using the FMD non structural protein ELISA to determine recently infected FMD cases, thereby estimating the seroprevalence in district or zone.

Sample frame

A total of 352 serum samples were aseptically collected from buffaloes and cattle of eight towns of Faisalabad. Random selection method was used for the sampling of animals. The total numbers of samples were equally collected from the towns of Faisalabad. Sample size for the seroprevalence of FMDV will be calculated with the help of following formula as described by Thrusfield (18).

$$n = \frac{1.96^2 P \exp (1- P \exp)}{d^2}$$

Where 'n' is number of samples, 'P_{exp}' is expected prevalence and 'd' is desired absolute precision. Expected prevalence was kept at 25% and desired absolute precision at 5%.

The serum samples were tested using the Non-Structural Protein (NSP) ELISA to determine the seroprevalence of FMD in district Faisalabad.

Measurement of FMD non-structural antibodies

The sera samples were tested using FMD 3ABC-ELISA SVANOVIR® kit (Svanova Diagnostic, Sweden) as the procedure described by manufacturer. Briefly the test sera, negative and positive control sera were diluted and added in duplicate to the wells of a 96 well microtitre plate pre-coated with the vector expressed viral 3ABC antigen. Following 30 min incubation at 37°C, plates were washed thrice with washing buffer. A horseradish peroxidises (HRP) conjugated anti-ruminant antibodies were added, plates

were sealed and incubated for 30 min. After another washing, tetramethyl benzidine (TMB) substrate was added and plates were incubated at room temperature for another 30 min in dark conditions. The reaction was terminated by adding 1M sulphuric acid as stopping solution. The optical density (OD) of the samples was measured at 405 nm with a Biotek® (USA) ELISA reader and the result was expressed as an index derived by dividing the corrected OD value of the test serum by that of corrected OD value of positive control. A sample with OD \geq 48% was considered positive and OD $<$ 48% was negative (16).

Data analysis

The data were sorted in Microsoft Excel Spreadsheet and statistical analysis were computed using Minitab software version 16.1 (Minitab Inc, State College, PA, USA). Chi Square test and odd's ratios were employed to detect any association of risk factors with that of Foot and Mouth Disease infection.

RESULTS & DISCUSSION

During the study period 352 animals were examined for the presence of antibodies against Foot and Mouth Disease Virus (FMDV) in their blood samples using (NSP) ELISA test. The overall seroprevalence of FMD in Faisalabad (28.40%) (100/352) is an indication of its importance in the study area. The seroprevalence documented in this study showed high value (22.81%) in Faisalabad when compared to the previous reports of Anjum *et al.* (3) and low (33.2%) as compared to a recent study of Abubakar *et al.* (2). The variation in seroprevalence may be due to the changing pattern of disease or gap in the vaccination schedule.

Geographical seroprevalence

Seroprevalence of FMD in different towns of district Faisalabad was highest (38.63%) in Tandlianwala town and lowest (18.18%) in Jinnah town (Fig.1). Statistically there was no significant difference between the towns of Faisalabad ($p>0.05$).

Species & sex seroprevalence

A significant difference ($p<0.05$) in seroprevalence of FMD was found between cattle (40%) and buffalo (20.75%) which was nearly supported by the observation of Abubakar *et al.* (1) on the prevalence of FMD which was 64.00% in cattle and 34.00% in buffaloes of Pakistan. This might be due introduction of exotic blood and cross breeding in cattle population at large

scale, which increases vulnerability of cattle to FMD (20). In contrast Awan *et al.* (4) reported 10.3% susceptibility in buffaloes and 9.9% in cattle of Punjab.

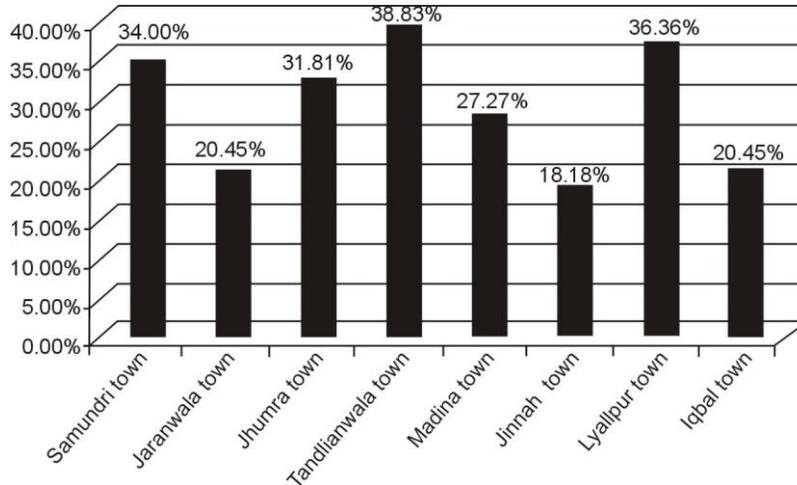


Fig. 1. Seroprevalence of FMD in different towns of Faisalabad.

In females the seroprevalence was noted to be 30.27% whereas in males it was 25.37%. The percentage is higher in females but statistically there was no significant variation in the seroprevalence of FMD in females and males ($p>0.05$) (Table I). This finding was consistent with the previous findings i.e. 15.7% in female and 8.27% in male (9) and 67% in female and 33% in male (7) in Ethiopia and Kenya respectively, where sex apparently does not have a significant effect on seropositivity of Foot and Mouth disease. The greater percentage of seropositivity in females might be due to the physiological stresses which include oestrus, pregnancy and lactation which are known to affect females resistance to infection (18). These results are contrary to those of Awan *et al.* (4), Megersa *et al.* (16) and Mohamoud *et al.* (17).

Table 1. Seroprevalence of FMD in buffaloes and cattle according to species and sex in Faisalabad.

Species wise seroprevalence				Sex wise seroprevalence			
Species	Buffal	Cattle	Total	Sex	Male	Female	Total
Animal sampled	212	140	352	Animal sampled	134	218	352
Animal positive	44	56	100	Animal positive	34	66	100
Seroprevalence %	20.75	40.00	28.40	Seroprevalence %	25.37	30.27	28.40
P- Value	0.004			0.458			

Seroprevalence according to Pregnancy and herd structure

There was no role of pregnancy status in the prevalence of the disease as seroprevalence was found to be 34% in pregnant animals and 28.88% in non pregnant which have non-significant difference (Table 2).

Table 2. Seroprevalence of FMD in buffaloes and cattle according to pregnancy in Faisalabad.

Seroprevalence according to pregnancy			
Pregnancy status	Pregnant	Non Pregnant	Total
Animal sampled	50	90	140
Animal positive	17	26	43
Seroprevalence %	34.00	28.88	30.71
Odd's Ratio	1.17		
P- Value	0.649		

Seroprevalence of FMD was 16% in herds having only large ruminants whereas 31.76% in herds having large as well as small ruminants. These findings are supported by the results of Gelaye *et al.*, Lazarus *et al.*, and Megersa *et al.*, (9, 13, 15) which showed that sheep and goats are important reservoirs for FMD infection in cattle and buffaloes. The difference in seroprevalence in mixed herd type was found significant and statistically associated with the disease (Table 3).

Table 3. Seroprevalence of FMD in buffaloes and cattle according to herd type in Faisalabad.

Seroprevalence according to herd type			
Herd type	Large ruminants	Large and small (mix)	Total
Animal sampled	75	277	352
Animal positive	12	88	100
Seroprevalence %	16.00	31.76	28.40
Odd's Ratio	1.99		
P- Value	0.037		

Seroprevalence according to Age

There was significant variation in seroprevalence among different age groups. The susceptibility rate was highest (36.84%) in animals of >4 years age and lowest (20.71%) in animals of <2 years age. The higher seroprevalence of disease in young and adult animals as compared to calves observed in the current study is in line with the previous reports of Awan *et al.* (4), Gelaye *et al.* (9), Megersa *et al.* (16) and Mohamoud *et al.* (17), Mannan *et al.* (14) and Chepkwony *et al.* (7). On the other hand Esayas *et al.*, (8) reported non-significant association between seropositivity of FMD and age of cattle in Ethiopia. The low seroprevalence of FMD recorded in young calves could be associated with the low frequency of exposure. Whereas, increased susceptibility in old cattle might be due to malnutrition, poor management system and poor immunity. There was concomitant variation between age of animals and prevalence of the disease (Table 4).

Table 4. Seroprevalence of FMD in buffaloes and cattle according to age in Faisalabad.

Age (years)	Young (<2 years)	Adult (2 - 4 years)	Old (> 4 years)	Total
Animals sampled	140	98	114	352
Animals positive	29	29	42	100
Seroprevalence (%)	20.71	29.59	36.84	28.40
P-value	0.033			

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