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EPIDEMIOLOGY OF FOOT AND MOUTH DISEASE IN BUFFALOES AND CATTLE OF PUNJAB USING NON STRUCTURAL PROTEINS ELISA.

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Sero-epidemiological study was conducted in three districts of Pakistan (Chakwal, Faisalabad and Khanewal) from November 2011 to October 2012 in buffaloes and cattle. Total 900 serum samples were collected from buffaloes (n= 480) and cattle (n= 420) having no history of Foot and Mouth Disease within the study area. The sera were examined using 3ABC (Non Structural Proteins) Enzyme Linked Immunosorbent Assay (ELISA). Results of the current study showed that the overall seroprevalence of FMD in bovine was (19.33%) with 16.04% in buffaloes and 23.09% in cattle. Significantly higher (P= 0.000) seroprevelance was recorded in Faisalabad vicinity (29.33%) as compared to the Khanewal (17.66%) and Chakwal (11%) districts. From the various risk factors analysed age, species and herd type were found to be statistically ($P<0.05$) associated with the disease whereas sex and pregnancy did not have any association with the disease. The higher seroprevalence of disease has substantial economic implications which signify the need for devising effective control measures.

Keywords: Apthovirus, epidemiology, FMD, NSP–ELISA, risk factors, livestock diseases

INTRODUCTION

Foot and Mouth disease (FMD) is a highly contagious disease affecting primarily cloven footed animals, continues to be a major concern for world livestock industry. FMD is a viral disease caused by genus Apthovirus of family *Picornaviridae*. There are seven serotypes of FMD virus i.e. A, O, C, Asia-1, SAT-I, SAT-II, and SAT-III. In Pakistan, the most prevalent serotypes are O (70%), Asia-I (25%) and A (4.67%) causing a loss of more than Rs. 6.00 billion to farmers annually (Zulfiqar, 2003). The disease is characterized by fever and vesicular eruptions in mouth, nares, muzzle, foot, teats and other hairless soft areas of body (Choudhury *et al.,* 1994).

Although FMD does not cause high mortality in adult animals, the disease has debilitating effect on animal health which includes reduced milk production, loss of drought power, decrease in body weight and reproductive failure. FMD virus causes myocardial degeneration in young animals, known as Tiger Heart Disease which causes mortality (Gleeson *et al*., 2003). The infected animals can excrete virus rapidly before the onset of clinical signs and at the time of disease development excretion reaches at its peak (Alexandersen *et al*., 2003). Tests for antibodies to some non-structural proteins of FMD virus are valuable in providing confirmation of previous or current viral replication in the host, despite of vaccination status. The non-structural proteins unlike the structural proteins are highly conserved and are not serotype specific. Since the inactivated vaccine in FMD used partially purified virus antigen (free of NSP), antibody response to NSP in a non-vaccinated cattle serum is indicative of an infection status rather than response to vaccination (Diego *et al*., 1997). The development of ELISA tests against 3ABC (NSP) has greatly enhanced the serosurveillance of FMD as they detect exposure to live virus for all the seven serotypes of virus even in vaccinated herds (Bronsvoort *et al*., 2006).

In Pakistan there is need for regular and continuous surveillance of the disease, identification of its associated risk factors because more than 50% of the cattle and buffalo population of Pakistan is residing in Punjab province. As the bovine population of Punjab has never been investigated for seroprevalence by this technique, so the present study was designed to determine the seroprevalence of FMD by identifying antibodies against Non Structural proteins through ELISA and identification of certain risk factors associated with the disease.

MATERIALS AND METHODS

***Study area*:** As there was no previous data on seroprevalence of FMD in Punjab, the current study was carried out in three districts of Punjab, Pakistan including Khanewal, Faisalabad and Chakwal representing the south, central and north portion of Punjab province respectively. These districts were selected due to diversity in their climatic conditions, average rainfall, height above sea level and livestock population as well as district Faisalabad and Khanewal have canal irrigated lands while district Chakwal have Arid agricultural land.

***Study design and sample estimation*:** A cross sectional survey was conducted and a questionnaire was designed to collect information on individual herds from the owners. Risk factors such as age, sex, species, herd type and pregnancy status were recorded. On the basis of age, animals were divided into three groups (<2 years, 2-4 years and >4 years). A total of 900 serum samples were collected from three selected districts. Sample size for the seroprevalence of FMD virus in each district was calculated with the help of following formula as described by Thrusfield (2007):

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

Where n is number of samples, P_{exp} is expected prevalence and d is desired absolute precision. Expected prevalence was kept at 25% (Anjum *et al*., 2006) and desired absolute precision at 5%.

***Collection of serum samples*:** A total of 10 ml blood sample was aseptically collected from jugular vein of cattle and buffalo using vacutainer tubes and an identification code was given to each sample. The blood samples were allowed to stand at room temperature for serum separation. The sera were shifted to serum tubes and then kept at -20°C till further processing.

***Measurement of FMD non-structural antibodies*:** The sera samples were tested using FMD 3ABC-ELISA SVANOVIR®kit (Svanova Diagnostic, Sweden) at Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan using the procedure as described by kit manufacturer. Briefly the serum samples, negative and positive controls were added to 96 well ELISA plates coated with FMD 3ABC antigen. Following 30 min incubation at 37°C, plates were washed 3 times with washing buffer after which Horse Radish Peroxidase (HRP) conjugated anti-ruminant antibodies were added to the plate and incubated for another 30 min at 37°C. After washing, Tetramethyl Benzidine (TMB) substrate was added and plates were incubated at room temperature for 30 min in dark. The reaction was terminated by adding 1M sulphuric acid as stopping solution. The optical density (OD) of the samples was measured at 405 nm in Biotek® USA ELISA reader and the results were calculated as described by Megersa *et al.* (2009).

***Data analysis*:** The data was stored in Microsoft Excel Spreadsheet. Analytical statistics were computed using Minitab software. Chi Square test and odds ratio were employed to detect any association of risk factors with that of Foot and Mouth Disease infection (Wardlaw, 1985).

RESULTS

The overall seroprevalence of FMD in study area was 19.33% (174/900). Seroprevalence of FMD was varying significantly ($p<0.05$) in three regions of the Punjab province. It was highest in Faisalabad (29.33%) followed by Khanewal (17.66%) and Chakwal (11%) districts (Table 1).

***Species based distribution*:** Seroprevalence of FMD was significantly higher ($p<0.05$) in cattle (23.09%) than in buffaloes (16.04%) (Table 1).

***Sex based distribution*:** Seroprevalenceof FMD in females and males of cattle was 24.20% and 21.42% while in buffaloes it was 17.22% in females and 13.42% in males respectively. Statistically there was no significant variation in the seroprevalence of FMD in females and males of both the species ($p>0.05$) (Table 2).

***Age based distribution*:** There was significant variation in seroprevalence between different age groups ($P< 0.05$). The rate of susceptibility was highest (23.43%) in animals of >4 years age and lowest (13.33%) in animals of <2 years age. There was concomitant variation between prevalence of the disease and age of animals (Table 3).

***Herd based distribution*:** Seroprevalence of FMD was significantly higher in herds having large as well as small ruminants (21.57%) as compared to herds having large ruminants only (11.50%) (Table 4).

***Pregnancy based distribution*:** Pregnancy status was not found to have any role in the prevalence of the disease. Seroprevalence was 35.00% in pregnant animals and 28.57% in non pregnant. The difference was non-significant ($p>0.05$) (Table 5).

Table 1. Comparative seroprevalence of FMD in buffaloes and cattle in three districts of Punjab

Location	Buffalo		Cattle		Total (Prev. %) Positive/Sampled
	Positive/Sampled	Prevalence %	Positive/Sampled	Prevalence %	
Chakwal	12/160	7.50	21/140	15.00	33/300 (11.00)
Faisalabad	40/160	25.00	48/140	34.28	88/300 (29.33)
Khanewal	25/160	15.62	28/140	20.00	53/300 (17.66)
Total	77/480	16.04	97/420	23.09	174/900 (19.33)
		Significant (P<0.05)			
Area wise = χ^2=22.072 (df =2), P=0.000			Species wise= χ^2=4.816 (df =1), P=0.028		

Table 2. Comparative Sex wise seroprevalence of FMD in buffaloes and cattle in three districts of Punjab

Location	Buffalo		Cattle	
	Male (Prev. %)	Female (Prev. %)	Male (Prev. %)	Female (Prev. %)
Chakwal	4/47 (8.51)	8/113 (7.07)	4/45 (8.88)	17/95(17.89)
Faisalabad	11/51 (21.56)	29/109 (26.60)	19/61(31.14)	29/79 (36.70)
Khanewal	5/51 (9.80)	20/109 (18.34)	13/62 (20.96)	15/78 (19.23)
Total	20/149 (13.42)	57/331 (17.22)	36/168 (21.42)	61/252 (24.20)
		Non Significant (P>0.05)		

χ^2=0.594 (df =1), P=0.441

Table 3. Comparative age wise seroprevalence of FMD in buffaloes and cattlein three districts of Punjab

Animal Species	Sampled (Age group distribution)	Age categories		
		< 2 years (Prev. %)	2-4 years (Prev. %)	>4 years (Prev. %)
Buffalo	480 (180,130,170)	23 (12.77)	22 (16.92)	32 (18.82)
Cattle	420 (150,120,150)	21 (14.00)	33 (27.50)	43 (28.66)
Total	900(330,250,320)	44 (13.33)	55 (22.00)	75 (23.43)
		Significant (P<0.05)		

χ^2=8.438 (df =2), P=0.015

Table 4. Comparative herd type wise seroprevalence of FMD in buffaloes and cattle in three districts of Punjab

Location	Type of Herd				Odds Ratio
	Buffaloes and Cattle		Kept with small ruminants (mixed)		
	Sampled	Positive (Prev. %)	Sampled	Positive (Prev. %)	
Chakwal	61	5 (8.19)	239	28 (11.71)	
Faisalabad	71	11 (15.27)	229	77 (33.62)	
Khanewal	68	7 (10.29)	232	46 (19.82)	1.870
Total	200	23 (11.50)	700	151 (21.57)	
		Significant (P<0.05)			

χ^2=7.185 (df =1), P=0.007

Table 5. Comparative Seroprevalence of Foot and Mouth Disease in three districts of Punjab on the basis of pregnancy status

Area Sampled	Buffalo				Cattle				Odds Ratio
	Pregnant		Non-pregnant		Pregnant		Non-pregnant		
	Sampled	Pos (%)	Sampled	Pos (%)	Sampled	Pos (%)	Sampled	Pos (%)	
Chakwal	5/23	21.73	14/45	31.11	7/16	43.75	10/42	10 (23.80)	
Faisalabad	6/20	30.00	20/52	38.46	10/23	43.47	9/39	9 (23.07)	
Khanewal	6/17	35.29	14/50	28.00	8/21	38.09	13/52	13 (25.00)	
Total	17/60	28.33	48/147	32.65	25/60	41.66	32/133	24.06	1.225
				Non Significant (P>0.05)					

χ^2=0.856 (df =1), P=0.355

DISCUSSION

The devastating economic losses caused by FMD are due to death of young animals, marked reduction in milk production, abortion at an advanced stage of pregnancy and reduced working ability of the animals (Singh, 2003) along with reduced quality and quantity of meat, reduction in fertility, loss of quality of semen in breeding bulls and loss of productivity for a considerably longer time (Yadav, 2003).

The overall seroprevalence (19.33%) recorded for FMD in this study is an indication of its importance in the study area. The seroprevalence documented in this study showed high value when compared to the previous reports of Hafez *et al*. (1994) which was 16% in Saudi Arabia, 12.8% by Gelaye *et al*. (2009), 14.05% by Mohamoud *et al.* (2011) in Ethiopia

and 17.6% by Dukpa *et al.* (2011) in Bhutan. On the other hand, the seropositivity findings of this survey was lower than the overall seroprevalences of 61% and 72.62% reported by Mwiine *et al.* (2010) and Lazarus *et al.* (2012) in Uganda and Nigeria, respectively. At a district level, significantly ($P<0.05$) higher seroprevalence was observed in Faisalabad 29.33% than Khanewal 17.66% and Chakwal 11% which was close to the findings of 22.81% prevalence in Faisalabad and 10% in Chakwal districts. This probably relates to difference in livestock population in Punjab as well as congested population in Faisalabad and Khanewal areas (Anjum *et al*., 2006).

The seroprevalence of FMD in male and females of both buffaloes and cattle in this study was 13.42% in males and 17.22% in females of buffaloes while 21.42% in males and 24.20% in females of cattle. This finding was consistent with the previous findings in Ethiopia 8.27% in male and 15.07% in female by Gelaye *et al*. (2009) and 33% male and 67% female by Chepkwony *et al.* (2012) in Kenya. The greater percentage in females might be due to thephysiological stresses which include oestrus, pregnancy and lactation which are known to affect their resistance to infection (Susan and Asamays, 1998). On the contrary, Megersa *et al.* (2009) and Mohamoud *et al.* (2011) in their reports on seroprevalence of FMD documented a higher rate in male than that of female (10.1 % males and 9.2% in females) and (19.4% males and 13.6% females) in Ethiopia.

A significant ($p<0.05$) difference in species susceptibility was found in buffalo (16.04%) and cattle (23.09%) which was supported by the observation of Abubakar *et al*. (2009) on the prevalence of FMD which was34.00% in buffaloes and 64.00% in cattle of Pakistan. 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 study revealed a significant variation on sero-positivity of foot and mouth disease among the three age groups. The significantly higher seroprevalence of FMD in young and adult animals than in claves observed in the current study is in agreement with the previous reports of Gelaye *et al*. (2009), Megersa *et al.* (2009) and Mohamoud *et al.* (2011) in Ethiopia, Mannan *et al.* (2009) in Bangladesh and Chepkwony *et al.* (2012) in Kenya. On the other hand (Esayas *et al.,* 2009) done their research in Bench Maji zone of southern Ethiopia documented no significant association between seropositivity of FMD and age of cattle. The low seroprevalence of FMD recorded in young claves could be associated with low frequency of exposure to disease. In addition, farmers keep their young calves near homestead and away from the grazing adult animals (Thrusfield, 2007).

The seroprevalence of FMD was 11.50% in herds having only large ruminants and 21.57% in herds having large as well as small ruminants (mixed). In previous studies, it has been shown that small ruminants are important reservoirs for FMD infection in cattle and buffaloes (Gelaye *et al*., 2009; Megersa *et al*., 2009). The results showed significant ($p<0.05$) difference between both groups.

***Conclusion*:** In conclusion, the seroprevalence of FMD was found to be high in Faisalabad and Khanewal districts of Punjab. The age, species and herd type remains the major risk factors for the occurrence of Foot and Mouth Disease in buffaloes and cattle. Therefore, a massive vaccination plan should be applied in Punjab with special consideration to cattle population and rearing of mixed herd should be avoided to control the economic impact of the disease.

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