# SEROPREVALENCE OF FOOT-AND-MOUTH DISEASE AND PROTECTIVE ANTIBODY TITRE AGAINST IT IN BUFFALOES

**Anand Mohan[1*], Ajay Kumar Upadhyay[1], Mahesh Kumar[1], Neelam Kushwaha[2], Rashmi Singh[3] and Sunil Kumar Rastogi[4]**

## ABSTRACT

Liquid phase blocking (LPB) ELISA is approved for the screening of sera samples after vaccination and non-structural protein (NSP) ELISA for confirmation of infection regardless of vaccination status of animals. In the present study sera samples from 300 buffalo were used to screen for both protective and NSP antibody titre against foot-and-mouth disease (FMD) virus. Overall, 6.67% buffalo were infected with FMD whereas 10.67% had suspicion level of titre against the disease. However, protective antibody titre against O, A and Asia-1 serotypes were respectively, 62.67%, 20.00% and 48.00%. Among the breeds, a greater number of Murrah had protective antibody titre against different serotypes of FMD virus (FMDV) namely O, A and Asia-1 than other breeds of buffalo. Comparison of protective antibody titre and doubtful titre of NSP antibody between male and female showed that the proportion of female buffaloes had higher in both protective antibody and titre against NSP in doubtful status. However, no cases of infection were detected in male animals but 9.62% female buffalo were infected with FMD. Similarly, in the age groups of 0 - <4 years and ≥8 years, no cases of FMD infection were established, but 8.20% buffaloes in the age group of 4 - <8 years was infected. Proportion of buffaloes that had protective antibody titre was highest in the age group of 0 - <4 years followed by 4 - <8 years and ≥8 years. Breed, sex and age of buffaloes influenced the protective antibody titre ($p<0.01$) against different serotypes of FMDV except for serotype A in breeds and age groups of animals ($P>0.01$).

**Keywords:** buffalo, FMD, LPB ELISA, NSP ELISA, seroprevalence

## INTRODUCTION

FMD is a highly contagious and sometimes fatal disease of cloven-hoofed animals such as cattle, buffalo, sheep, goats etc. Seven serotypes of FMDV are responsible for FMD outbreaks; however,

[1]Department of Epidemiology and Preventive Medicine, College of Veterinary and Animal Science, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, [*]Email: dr_rinkuanand@rediffmail.com

[2]Division of Veterinary Medicine, Indian Veterinary Research Institute, India

[3]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhyaya Pashu Chikiitsa Vigyan Vishwa Vidyalaya Evam Go Anusandhan Sansthan, Mathura, Uttar Pradesh, India

[4]Department of Veterinary Physiology and Biochemistry, College of Veterinary and Animal Science, Govind Ballabh Pant University of Agriculture and Technology, Uttarakhand, India

only serotypes A, O, and Asia-1 are prevalent in India. 100% morbidity due to this disease has been reported (OIE, 2007), but the case fatality rate does not exceed 5%. Higher fatality rates (up to 90%) have been observed in animals below 3 months of age due to cardiac muscle involvement (Gulbahar *et al.,* 2007).

Protective immune response to FMDV in animals is heavily dependent on humoral antibodies (McCullough *et al.*, 1992) and hence serum antibody titre is an indicator of the immune status/prevalence of disease in any geographical region. In developing countries including India where the disease is endemic, serological investigations assume more significance. Although serology is not the method of choice for the primary diagnosis of FMD, it can be of immense help in retrospective studies as the protective antibodies against FMDV persist for 4 to 6 months post-infection. Serological assays with typing of field isolates can give a precise and more accurate picture of the disease topography. Apart from that, serological monitoring is an important component of FMD control programme and epidemiological surveillance.

The serum neutralization test, sandwich ELISA, sandwich competition ELISA, liquid phase blocking ELISA and liquid phase blocking competition ELISA were used for monitoring the antibody response in domestic animals after vaccination but competitive ELISAs particularly blocking ELISA were the most effective as far as efficacy is concerned (McCullough *et al.,* 1992; Saha and Sen 1995; Araujo *et al.,* 1996; O'Donnell *et al.,* 1996; Mousa and Youssef 1998). Niedbalski *et al.* (1994) described LPB sandwich ELISA for serological study of antibody to FMDV. They found that assay was sensitive, specific, precise and useful for large-scale serological screening. It was easy and rapid to perform and the results could be obtained after one day.

The demonstration of specific antibodies to structural proteins in unvaccinated animals is indicative of prior infection with FMDV. Tests for antibodies to some non-structural proteins (NSPs) of FMDV are useful in providing evidence of previous or current viral replication in the host, irrespective of vaccination status. NSPs, unlike structural proteins, are highly conserved and therefore, are not serotype specific and as a consequence, the detection of these antibodies is not serotypes restricted (Kweon *et al.,* 2003). Different non-structural proteins (NSP) of FMDV were evaluated for diagnostic potential in ELISA **(**Paton *et al.,* 2006; Campos *et al.,* 2008**)**. Non-structural-polypeptide 3ABC was found to be the most reliable marker of infection (De Diego *et al.,* 1997; Clavijo *et al.,* 2004). In addition, antibodies to 3ABC appeared earlier during infection and were detected for longer periods than antibodies to any other NSPs examined (Mackay *et al.,* 1998).

Based on this background, the present study was designed to screen buffalo sera from a border region adjacent to both Uttarakhand and Uttar Pradesh state (India) to monitor the protective antibody titre in serum against FMD by LPB ELISA and true seroprevalence of FMD by NSP ELIA.

## MATERIALS AND METHODS

### Sample collection and procurement of reagents

A total of 300 blood samples of buffaloes from the border area between Uttarakhand and Uttar Pradesh, India were collected aseptically by jugular venepuncture in vacutainers. Details of samples in respect to species, sex and age are given in Table 1. Sera were separated from blood samples by placing tubes in slanting position for

1 h at room temperature followed by 4 h at 4°C. Thereafter, tubes were centrifuged at 3000x g for 5 min. A clear supernatant on top of tubes were collected and stored in aliquots at -20°C in the deep freeze till use.

Reference control antigens of FMDV types O, Asia-1 and sub-type $A_{22}$; anti-146S sera raised in rabbit and guinea-pig against the reference type O, Asia-1 and sub-type $A_{22}$; Prokaryotic expressed recombinant 3AB3 NSP; positive serum; negative serum; anti-bovine HRPO conjugate and anti-guinea pig HRPO conjugate were obtained from the Central FMD Virus Typing Laboratory, Mukteshwar, India.

**Liquid Phase Blocking Enzyme Linked Immunosorbent Assay**

Rabbit serum and guinea pig serum against 146S antigen were used as coating sera and tracing sera, respectively. Coating antiserum was used in dilutions of 1:4000 (Type O), 1:5000 (A) and Asia-1 (1:10000). Reference LPB Antigens were used in dilution of Type O (1:3), Type A (1:3) and Type Asia-1 (1:10), respectively. The tracing antibody Type O, A and Asia-1 were used in dilutions of 1:4000, 1:5000 and 1:1000, respectively, whereas anti-guinea pig HRPO conjugate was used in dilution of 1:3000. Liquid phase sandwich blocking ELISA was performed as described by McCullough *et al.* (1992). Titre of different wells estimated as: reciprocal $\log_{10}$ dilution corresponding to 50 percent inhibition in optical density with respect to antigen control wells. Percentage inhibition of each well was calculated in relation to antigen control using the following formula, thus titre obtained ≥1.8 for samples was interpreted as protective for the concerned serotype while samples giving <1.8 titre were taken as not protective for the concerned serotype(s) of FMD.

**Non-Structural Protein Enzyme Linked Immunosorbent Assay**

Polystyrene immuno plates (Nunc, Maxisorp) were coated with 3AB3 recombinant protein (50 ng/well) diluted in carbonate-bicarbonate buffer (0.2 M, pH 9.6) for overnight at 4°C. Plates were washed three times without holding time with washing buffer (2.5 mM, dihydrate sodium dihydrogen phosphate; 2.93% w/v, NaCl; 0.05% w/v, Tween-20; pH 7.2-7.4). Meanwhile, in low binding perpex plates negative and positive control serum were diluted respectively, 1:20 and 1:10 in diluents buffer (3% w/v, skimmed milk powder; 10% v/v, chicken serum; 0.01% v/v, *E. coli* lysate in washing buffer). In duplicate, 100 µl diluted test serum, positive serum and negative serum were transferred to coated plates. For background control only 100 µl of diluents buffer was dispensed without any serum and incubated at 37°C for 1 h. Antigen-antibody complexes were traced by anti-bovine-HRP conjugate, diluted in diluents buffer (1:2000) and used as 50 µl/well incubated for 1 h at 37°C. Fresh prepared substrate solutions, 50 µl/well were used for development of color at 37°C without shaking for 15 min. Reactions were stopped by 1 M $H_2SO_4$, 50 µl/well. Optical density of plates was taken at 492 nm.

The tests were considered valid as mean absorbance of the positive control wells were 1.0 with ±20% coefficient of variation, mean absorbance of negative control serum were <0.4 and OD of background control were between 0.00 to 0.05. Test serum was considered positive if T/P ratio was more than 0.5, under suspicion if T/P ratio fell in between 0.4-0.5 and negative if it was less than 0.4.

**Statistical analysis**

Analyses of data were done with software downloaded from the website www.spss.com. The chi square test was applied on data at the 1% level of significance. Pearson chi-square values thus obtained were compared with chi square table values at the 1% level of significance. Pearson chi-square values more than 0.01 were taken as non-significant for the respective category.

## RESULTS

The immune system of animals produces antibody against invading pathogens so as to protect itself. Most immunological tests target these antibodies as indicators of either infection or immunity such as may be deliberately induced by vaccination. LPB ELISA was used here to monitor the immune status of buffaloes against different serotypes of FMDV. But they didn't differentiate whether the titre produced in an animal was due

Table 1. Samples collected in different category of buffalo along with number of positive attributes diagnosed by LPB and NSP-ELISA. Figure in parenthesis represent number of buffalo in respective groups; *Diagnosed buffalo had antibody titre due to infection without discrimination of any serotype(s) involved; **Determine protective antibody titre in animals against different serotypes of FMDV.

| Animal Category | | | NSP ELISA* | | LPB ELISA**(Serotypes) | | |
|---|---|---|---|---|---|---|---|
| **Age** | **Sex** | **Breed** | **Infection** | **Doubtful Status** | **O** | **A** | **Asia-1** |
| 0 - <4 yrs (24) | Male (0) | Murrah (0) | - | - | - | - | - |
| | | Others (0) | - | - | - | - | - |
| | Female (24) | Murrah (16) | - | - | 16 | 8 | 12 |
| | | Others (8) | - | - | 4 | 0 | 8 |
| 4 - <8 yrs (244) | Male (92) | Murrah (84) | - | - | 36 | 8 | 8 |
| | | Others (8) | - | 4 | 4 | 0 | 8 |
| | Female (152) | Murrah (76) | 16 | 20 | 76 | 24 | 52 |
| | | Others (76) | 4 | - | 40 | 12 | 44 |
| ≥8 yrs (32) | Male (0) | Murrah (0) | - | - | - | - | - |
| | | Others (0) | - | - | - | - | - |
| | Female (32) | Murrah (4) | - | - | 4 | 0 | 0 |
| | | Others (28) | - | 8 | 8 | 8 | 12 |
| Total (300) | Male (92) | Murrah (84) | - | - | 36 | 8 | 8 |
| | | Others (8) | - | 4 | 4 | 0 | 8 |
| | Female (208) | Murrah (96) | 16 | 20 | 96 | 32 | 64 |
| | | Others (112) | 4 | 8 | 52 | 20 | 64 |

to vaccination or infection. Therefore, NSP ELISA was incorporated in the study to differentiate between vaccination and infection. In the present study, 6.67% animals were positive for FMD, whereas 10.67% had antibody titre which was in doubtful condition. The proportions of animals having protective immunity were 62.67%, 20.00% and 48.00% respectively against serotype O, A and Asia-1 (Table 2).

Among the breeds of buffaloes, comparatively greater numbers of FMD cases were reported in Murrah (8.89%) than in other breeds of buffalo (3.33%). Similarly, doubtful cases of FMD in these groups were 11.11% and 10.00%, respectively. In addition, 73.33%, 22.22% and 40.00% Murrah were protective against, respectively, O, A and Asia-1. However, protective levels for these serotypes in other breed were 46.67%, 16.67% and 60.00%, respectively (Tables 1 and 2).

In male animals, no case of FMD infection was established; however, 4.35% of the male buffaloes under study had titre in doubtful condition. But in females, 9.62% of the buffaloes were infected with FMD and 13.46% were in doubtful condition. As far as immunity is concerned, comparativelygreater numbers of female buffaloes (71.15%, 25.00% and 61.34%) had protective antibody titre than male buffaloes (43.48%, 8.70% and 17.39%) against serotypes O, A and Asia-1,

Table 2. Proportion of positive animals out of total in LPB and NSP-ELISA respectively for protective antibody titre and infection status of buffalo in different breed, sex and age of animals; Figure in parenthesis represent number of buffalo in animal category column and proportion in other columns; * Diagnosed buffalo had antibody titre due to infection with any serotype(s) of FMDV; ** Determine protective antibody titre in animals against different serotypes of FMDV.

| Animal Category | NSP ELISA* | | LPB ELISA** (Serotypes) | | |
|---|---|---|---|---|---|
| | Infection | Doubtful Status | O | A | Asia-1 |
| Breed-wise seroprevalence | | | | | |
| Murrah (180) | 16 (8.89) | 20 (11.11) | 132 (73.33) | 40 (22.22) | 72 (40.00) |
| Others (120) | 4 (03.33) | 12 (10.00) | 56 (46.67) | 20 (16.67) | 72 (60.00) |
| Sex-wise seroprevalence | | | | | |
| Male (92) | - | 4 (04.35) | 40 (43.48) | 8 (08.70) | 16 (17.39) |
| Female (208) | 20 (09.62) | 28 (13.46) | 148 (71.15) | 52 (25.00) | 128 (61.54) |
| Age-wise seroprevalence | | | | | |
| 0 - <4 yrs (24) | - | - | 20 (83.33) | 8 (33.33) | 20 (83.33) |
| 4 - <8 yrs (244) | 20 (08.20) | 24 (09.84) | 156 (63.93) | 44 (18.03) | 112 (45.90) |
| ≥8 yrs (32) | - | 8 (25.00) | 12 (37.50) | 8 (25.00) | 12 (37.50) |
| Overall seroprevalence | | | | | |
| Total (300) | 20 (06.67) | 32 (10.67) | 188 (62.67) | 60 (20.00) | 144 (48.00) |

respectively (Table 1 and 2).

A peculiar trend was noticed regarding the protective antibody titre against serotype O and Asia-1 with age (Tables 1 and 2). The maximum number of animals in the younger age group, 0 - <4 years (83.33% and 83.33%) were immune to FMD serotypes O and Asia-1 followed by adult, 4 - <8 years (63.93% and 45.90%) and minimum in old, ≥8 years (37.50% and 37.50%). However, for serotype A, the maximum number buffaloes in age group 0 - <4 years (33.33%) were immune to infection followed by age groups ≥8 years (25.00%) and 4 - <8 years (18.08%). Regarding infection, no cases of FMD were established in age groups 0 - <4 years and ≥8 years but 8.20% of the buffaloes in age group 4 - <8 years were infected with FMD. Antibody titre in the age groups 0 - <4 years and ≥8 years showed that 9.84% and 25.00% buffalo had titre in doubtful condition.

**Statistical analysis**

Pearson chi-square value obtained by chi square test applied on data indicated that breed, sex and age of buffalo influenced the protective antibody titre ($p<0.01$) against different serotypes of FMD virus (FMDV) except for serotype A in which breed and age of animals ($P>0.01$) had no influence.

## DISCUSSION

India has a vast population of buffalo and these contribute substantially to the GDP in terms of milk and milk products (Annual Report, Department of Animal Husbandry, Dairying and Fisheries, 2012-13). A most common disease encountered in these animals is FMD since India is endemic for it. However, biannual vaccination somehow reduces the occurrence of the disease in buffaloes. Repeated vaccination along with regular exposure of virus to animals in endemic areas will induce antibody against it. Post-vaccination monitoring of antibody in domestic animals is regularly followed under the supervision of Project Directorate on Foot-and-mouth Disease (PDFMD). In the present study, screening of protective antibody titre against FMDV found that 62.67%, 20.00% and 48.00% of the buffaloes were immune to serotypes O, A and Asia-1, respectively. However, animals from other states had different protection levels for different serotypes of FMDV (Annual Report, Network Unit, Orissa, Project Directorate on Foot-and-Mouth Disease, 2008-09; Monika *et al.*, 2006; Pattnaik *et al.*, 2012; Tyagi *et al.*, 2009).

Overall, 6.67% buffalo was found to be infected whereas 10.67% were in doubtful condition. According to the Pattnaik *et al.* (2012), the average prevalence of FMD infection in bovine population based on anti-3AB NSP ELISA was found to be 27.5% (approx), whereas, in goats, sheep and pig the prevalence has been found to be 20.0, 15.0 and 2.0%, respectively. Similarly in different district of Haryana after the 8$^{th}$ phase of vaccination, NSP antibody in susceptible animals was lowered to 12.12% (Regional Research Centre on Foot-and-Mouth Disease, Hissar). A survey conducted on 32,000 cattle in different regions of India by PDFMD revealed an overall NSP seroprevalence of 31%, with ranges from 6-46% at the state level (Annual Meeting Report, OIE/FAO Reference Laboratories, 2009).

Analysis of protective and NSP antibody titre in respect to breed, age and sex of buffalo indicated that these have influence on the level of titre in different categories of animals. Similarly, Thomson *et al.* (1992) noticed difference in infection rate in different ages of African buffalo.

Hassanein *et al.* (2011) also noticed different level of infection in different age groups of animals. They also found differences in protective antibody titre in different age groups of animals.

In general, buffaloes are comparatively less susceptible to FMD (Hedger and Condy, 1985). The resistance in Indian buffalo might be due to continuous low level of FMDV exposure in endemic areas that might be responsible for development of protective antibody. In addition, buffaloes do not show clinical signs as much as cattle and so signs may not be seen. Within the buffalo population, it was noticed that the Murrah breed encountered comparatively high proportion of FMD cases in comparison to other breeds. Similar to other animals, a breed of buffaloes which has high production potential will encounter more stress and hence become susceptible to so many diseases including FMD. Regarding the age of animals and outcome of disease, it was established that it influenced FMD infections in animals (Bronsvoort *et al.,* 2008). The same may be applicable in the present significant differences among the different levels of infection and protection in buffalo. On the other hand, few reports are available regarding the effect of sex on disease outcome in FMD cases and other diseases of buffaloes.

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