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Serodiagnosis of Foot and Mouth Disease Virus in Buffalo Through Counter Immunoelectrophoresis and ELISA in Pakistan

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ABSTRACT

Counter immunoelectrophoresis (CIE) was performed primarily to screenout 120 serum samples against A, O and Asia-I types of FMD virus. The pooled antigen and pooled hyperimmune serum was taken as a positive control. It was recorded that 33 (28.3%) samples showed positive response through CIE. Further, it was indicated that the maximum percentage of positive samples were present in buffaloes of 7-9 years of age (50%). All the 33 samples, which were found positive against FMD types through CIE, were processed through ELISA for the antibody titration against three different types of FMD virus. It was found that 96.9, 93.9 and 54.54% samples showed positive response through ELISA using the FMD virus type O, A and Asia-I, respectively.

Key Words: FMD; Immunoelectrophoresis; Buffalo

INTRODUCTION

In the agro-based economy of Pakistan, Livestock Sector contributes major share in the total GDP. About 70% of milk and meat demand is fulfilled through the healthy cows and buffaloes. Cattle and buffalo population suffers from various diseases of infectious and non-infectious nature. Foot-and-mouth disease (FMD) has an economic significance, which is responsible for large economic losses in terms of morbidity through permanent loss of milk, meat and draughtability (Ajmal *et al.*, 1989).

Serological testing is an important component of any programme to control FMD. It is used in the epidemiological surveillance, vaccine testing and in the selection of an appropriate strain because of considerable intertypic variation that occurs in FMD virus types (Kruschke *et al.*, 1988). Several techniques have been cited in the literature to measure the FMD antibodies. Complement Fixation (CF) and Virus Neutralization (VN) tests have been frequently used for the detection of FMD virus antibodies. Both of these tests have problems related to either non-specific reactions, lack of simplicity and sensitivity, variability in the results and the need for specialized resources such as cell culture (Westbury *et al.*, 1988).

The present study may help to solve these constraints appreciably by the introduction of counter immuno-electrophoresis (CIE) and enzyme linked immunosorbant assay (ELISA) for the detection and titration of antibodies against FMD virus type A, O and Asia-I in buffaloes.

MATERIALS AND METHODS

Source of virus. The FMD virus types A, O and Asia-I were procured in the form of cell culture suspension from Veterinary Research Institute, Lahore and stored at –20°C for further use.

Collection and separation of serum. The blood samples from 120 buffaloes vaccinated one month prior to collection were collected from Govt. Livestock Farm, Haroonabad. These buffaloes were divided into four groups according to their age viz., 1-3, 4-6, 7-9 and 10-12 years. Serum was separated and heat inactivated at 56°C for 30 min. The samples were stored at –20°C for further use.

Preparation of hyperimmune serum. Twelve rabbits were divided into four groups viz., A, B, C and D, each comprising of three animals. Hyperimmune serum was raised in the rabbits of group A, B and C against virus type A, O and Asia-I, respectively, while group D was kept as negative control. Serum was collected and stored at –20°C for further use.

Purification of virus. Cell culture suspension of FMD virus types were separately purified by polyethylene glycol precipitation method as described by Minor (1988).

Determination of viral protein concentration. Protein concentration contained in each FMD virus type was estimated (Bradford, 1976). Bovine serum albumin (BSA) was used for the standard assay in spectrophotometer at 595 nm.

Counter immunoelectrophoresis (CIE). The test was conducted on horizontal gel electrophoresis system. The procedure for CIE was adopted with partial modifications in

the methods described by Kohn (1973), and Kohn and Mohan (1976). Cellulose acetate membrane (CAM) and agarose gel were used as two different running medium. Different interspot distances i.e. 1, 1.5 and 2 cm were maintained between the antigen and antibody. Finally, different electric currents were also applied i.e. 7, 5 and 2 mA across the wells.

Enzyme linked immunosorbent assay (ELISA). Method described used by Suresh *et al.* (1986) was followed while the coating of antigen was done following the method used by Frederick *et al.* (1990).

RESULTS AND DISCUSSION

The constraints in serodiagnosis of FMD virus types can be solved by the use of CIE, also it is more economical in terms of reaction and dilution of antigen (Centeno *et al.*, 1979). In the present study, three different types of purified FMD virus suspensions showed protein concentration of 89.6 µg, 51 µg and 32.25 µg in type O, A and Asia-I respectively. It was recorded that 33 (28.3%) samples showed positive response through CIE as detailed in Table I. Further, it was indicated that maximum percentage of positive samples were present in buffaloes of 7-9 years of age (50%), while a comparable response occurred at 20.45, 20.58 and 20% in the age group of buffaloes ranging from 1-3, 4-6 and 10-12 years, respectively (Table I).

Table I. Age wise distribution of positive cases through counterimmunoelectrophoresis vaccinated against FMD virus

Age of buffalo (Years)	Number of total samples	Number of positive samples	Percentage of positive samples
1-3	44	9	20.45
4-6	34	7	20.58
7-9	32	16	50.00
10-12	10	2	20.00

It was found that CAM gave rapid and clear visibility of precipitation bands within 30 min. While agarose gel medium did not give good results in spite of repeating four times, when different distances were maintained between the antigen and antibody spots. There was all together negative response recorded at 2 cm distance; whereas, positive response was obtained in the form of clear precipitation band at 1.5 cm as well as 1 cm distance apart. It was observed that the best response of precipitation bands were obtained at 2 mA current within 30 min, while satisfactory results were not obtained at the flow of higher current. It was evident from the present study that CIE proved efficient enough to screen out the positive samples and without requiring elaborate time to count the results. On CAM, the results of CIE may easily be read within 30 min at 24°C.

The significant contributions to the development of ELISA for the diagnosis of FMD made by Crowther and Elzein (1979), Hamblin *et al.* (1984) and Have *et al.* (1984) have been extended (Iqbal, 1993). Have *et al.* (1984) stated that maximum sensitivity and type specificity cannot both be achieved within the same diagnostic test but clearly they can be if the appropriate reagent and test formulation are selected. In the light of above facts, ELISA was performed on the positive serum samples screened through CIE to measure the antibody titers against virus type O, A and Asia-I. It was observed that only one sample was found to be negative against all three-virus types. In overall cases, the antibody titers ranged from 1:5 to 1:2027 for virus type O, 1:52 to 1:5684 for type A and 1:2 to 1:16 against type Asia-I (Table II). These results are comparable with Hamblin *et al.* (1987) who has reported the work on type FMV "O" isolated from sheep.

Table II. Antibody titers against FMD types through ELISA out of positive cases from counter immunoelectrophoresis

Sample No.	Antibody titres against FMD types using ELISA		
	O	A	Asia-1
1	1:351	1:789	-
2	1:391	1:368	-
3	1:445	1:2000	-
4	1:54	1:3895	1:14
5	1:702	1:1210	1:5
6	1:797	1:1578	1:16
7	1:54	1:1157	1:5
8	1:797	1:5684	-
9	1:378	1:52	-
10	1:513	1:4210	1:2
11	1:459	1:2105	-
12	1:445	1:2000	-
13	1:810	1:1157	1:14
14	1:67	1:2157	1:3
15	1:797	1:1578	1:16
16	1:54	1:1157	1:5
17	1:797	1:5105	-
18	1:378	1:1578	-
19	1:513	1:4210	1:2
20	1:716	-	-
21	1:378	1:2052	1:14
22	1:797	1:52	1:4
23	-	-	-
24	1:756	1:1894	-
25	1:810	1:2473	1:14
26	1:797	1:1842	1:5
27	1:445	1:1052	-
28	1:351	1:789	-
29	1:540	1:2210	-
30	1:54	1:3894	1:11
31	1:837	1:2789	1:14
32	1:2027	1:105	1:4
33	1:162	1:526	1:5

Keeping in view, the results of CIE and ELISA, it is suggested that CIE may be recommended for initial

screening to detect as many positive cases as possible. However, for declaring the exact antibody titers, a more reliable and specific test like ELISA may be applied, for sero-surveillance and disease control programme for FMD in the buffalo population.

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