INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2003/05–3–311–312
http://www.ijab.org

Molecular Characterization of Foot and Mouth Disease Virus Types A, O and Asia-I by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

IQBAL, M.S., S.U. RAHMAN, M.H. RASOOL AND M.K. MANSOOR
Department of Veterinary Microbiology, University of Agriculture, Faisalabad–38040, Pakistan

ABSTRACT

Polypeptide variations among locally isolated foot and mouth disease (FMD) virus types O, A and Asia-I were determined using SDS-PAGE technique. Cell culture suspension of virus type O and A segregated into seven protein bands, while in virus type Asia-I, the third band was missing. Three structural (capsid) proteins i.e. VP_2, VP_3, and VP_1 of 38.5, 33.5 and 16 KDa were present in all three types. The other visible bands represent non-specific or intermediate components. All the antigenic types of FMD virus under study may be resolved and well identified through this technique.

Key Words: Foot and Mouth disease virus; Molecular characterization; SDS-PAGE

INTRODUCTION

Livestock sector plays an important role in the economy of Pakistan. Beside other factors, animal diseases are important factor in lowering the productivity of the animals. Among the disease problems that prevent maximum exploitation of productivity in these animals, Foot and Mouth disease (FMD) has an economic significance, which is responsible for substantial losses in terms of lowered productivity, including loss of milk, meat and workability (Ajmal *et al.,* 1989).

There are seven serotypes of FMD virus i.e. A, O, C, Asia-I, SAT-I, SAT-II, and SAT-III but only A, O, C and Asia-I have been reported in the country (Afzal & Ellahi, 1966). The degree of difference among the serotypes is such that an animal which has been recovered from an infection with one virus type is still fully susceptible to the others, although, it is immune against re-infection to the same type or subtype.

In countries like Pakistan, where vaccination is carried out routinely, the assessment of types involved and advice given on the suitability of available vaccines is essential for disease containment and control. The present study reported the comparative antigenic behaviour of FMD virus type A, O and Asia-I, by sodium dodecyl sulphate polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Source of samples. Locally isolated vaccinal strains of 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.

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

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The technique for SDS-PAGE was followed as described by Laemmli (1970), with little modification necessary for the FMD virus types (Iqbal, 1993). The crude cell suspension of FMD virus type A, O and Asia-I were loaded on 12.5% gel plate adjusted at vertical electrophoresis system. Four protein markers i.e. bovine serum albumin (BSA), chicken egg albumin (CEA), pepsin and trypsin having 66, 45, 34.5 and 23.5 KDa molecular weights respectively were also loaded in the same gel. The gel was removed after an overnight period with 5 mA current, stained with silver stain at 37°C for 1 h (Wray *et al.,* 1981). The gel was destained in 70% acetic acid solution, dried and subjected to photography. Finally the molecular weights of the polypeptides were determined with the help of known molecular weights of protein markers (Weber & Osborn, 1969).

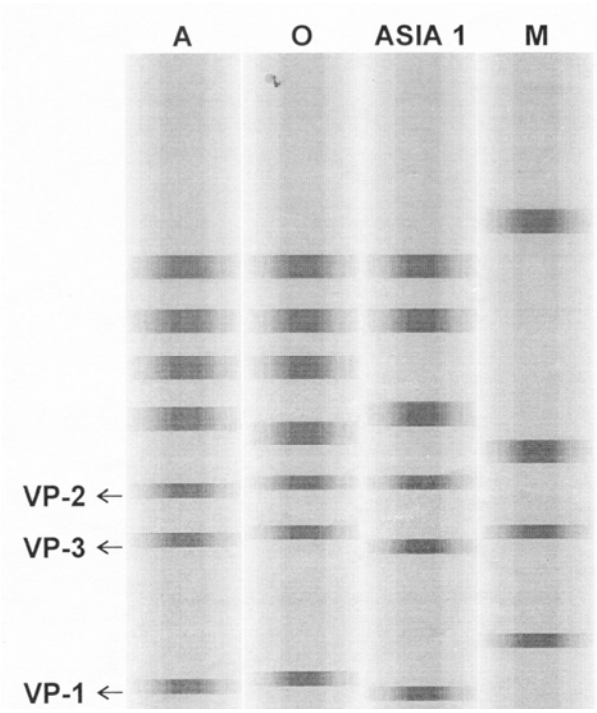
RESULTS AND DISCUSSION

In Pakistan, FMD virus serotyping was conducted on the basis of complement fixation text (CFT) by Afzal and Ellahi (1966), which was later reconfirmed to be the serotype A, O and Asia-I by Ajmal *et al.* (1989). The antigenic behavior of various FMD virus types have been studied through electrofocusing and oligonucleotide mapping (Kathryn *et al.,* 1979). Espinoza and Knowels (1983) have described Polyacrylamide gel electrophoresis to be extremely useful method for the study of FMD virus strains.

In the present study, results of SDS-PAGE revealed that cell culture suspension of FMD virus type O and A segregated into 7 bands while in the virus type Asia-I, the third band was missing. The position of the three structural (capsid) proteins i.e. VP_2, VP_3 and VP_1 were indicated

having molecular weights 38.5, 33.5, and 16 KDa respectively (Fig. 1). These capsid proteins are usually considered as the immunogenic proteins of FMD virus. Among these three, VP_1 is found to be the most immunogenic. The other visible bands of variable molecular weights represent non-specific or intermediate components of FMD antigen types (Sangar *et al.*, 1977).

Fig. 1. Polypeptide pattern of FMD virus type A, O and Asia-1 separated by SDS-PAGE



Analysis of the structural polypeptide by SDS-PAGE supported the serological findings that the three FMD virus types are not closely related. The results of the present study suggested that there is a sequence homology between the virus type O and A because of their similar polypeptide pattern in contrast to type Asia-I which has different polypeptide pattern. Although, it is difficult to correlate sequence homology between O and A, which is a measure of variation on the total RNA, to antigenic variation which is a reflection of difference in a maximum of 35-40 percent of RNA (Kathryn *et al.*, 1979).

It may be considered that there is a change among the virus type O, A and Asia-I with changes in RNA and structural polypeptide, reflecting the antigenic variations. Comparable results were obtained by Marquardt (1982) through gel-electrophoresis, who described that the antigenic variation among the FMD virus types was most likely the results of difference in amino acid sequence of at least one of the capsid proteins. Since capsid protein is encoded by the viral RNA, variation in the amino acid sequence must arise from differences in corresponding nucleic acid sequence. Furthermore, it is not yet known to what extent the genes for capsid protein and especially for non-capsid protein of FMD virus types differ from each other. Therefore, detailed biochemical analysis of FMD virus types, relating to the RNA structure, polypeptide composition and antigenicity in laboratory animals are required as it will provide some insight into the antigenic variation in this virus and ultimately helpful in the selection of the vaccine strains.

REFERENCES

Afzal, H. and A. Ellahi, 1966. A study of foot and mouth disease in Pakistan. *Bull. Int. Epizoot.*, 65: 101–10

Ajmal, M., M. Arshad and M.D. Ahmad, 1989. Coordinated Research entitled Epidemiology of Major Livestock Diseases in Pakistan (Pilot Study). *5th Annual Report,* 1989-90. Pakistan Agri. Res. Council, Islamabad, Pakistan.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Ann. Biol. Chem.*, 72: 248–54

Espinoza, A.M. and J.N. Knowles, 1983. A serological and biochemical study of new field isolates of foot and mouth disease virus type A in Peru, 1975-1981. *Vet Microbiol.*, 8: 555–62

Iqbal, M.S., 1993. Serodiagnosis of FMD virus in buffaloes through CIE and ELISA in Pakistan. *M.Sc. (Hons.) Thesis,* Dept. of Vet. Microbiol., Univ. Agri. Faisalabad, Pakistan.

Kathryn, J.H., Robson, Crowther, A.M.Q. King and F. Brown, 1979. Comparative Biochemical and serological analysis of five isolates of a single serotype of foot and mouth disease virus. *J. Gen. Virol.*, 45: 579–90

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature*, 227: 680–4

Marquardt, O., 1982. Three strains of European foot and mouth disease virus are highly conserved in the 3 termini and highly variable in the genes to two capsid proteins. *J. Gen. Virol.*, 59: 283–94

Sangar, D.V., D.N. Black, D.J. Rowlands and F. Brown, 1977. Biochemical mapping of foot and mouth disease virus genome. *J. Gen. Virol.*, 35: 281–97

Weber, K. and M. Osborn, 1969. The reliability of molecular weight determination by sodium dodecyl sulphate polyacrylamide gel electrophoresis. *J. Biol. Chem.*, 244: 4406

Wray, W., T. Boulikas, V.P. Wray and R. Hancock, 1981. Silver staining of proteins in polyacrylamide gels. *Ann. Biochem.* 118: 197–203

(Received 15 March 2003; Accepted 20 May 2003)