Comparison of some Type '0' Foot-and-mouth Disease Virus Isolates by Iso-Electrofocusing

E.M.E. Abu Elzeinl, J.W. Newman2 and B.J. Newman2

1 Department of Microbiology and Parasitology , College of Veterinary Medicine and Animal Resources, King Faisal University , P.O.Box 1757, Al-Ahsa 31982, Saudi Arabia.
2Institute for Animal Healths, Pirbright Laboratory, Pirbright Woking, Surrey, United Kingdom.

Abstract

Some Sudanese, Type '0', foot-and-mouth disease viruses were compared with standard Type '0' vaccine strains in Britain and Kenya, by isoelectric focusing The results obtained were correlated with previous serological and epidemiological data. The significance of isoelectrofocusing in differentiation of viruses was discussed.

Introduction

Foot-and-mouth disease (FMD) virus belongs to genus aphthovirus of the family picornaviridae (Cooper et al. 1978). Seven serotypes are known for FMDV. They are designated type 0, A, C, SAT-1, SAT-2, SAT-3 and Asia 1. Within each serotype there are subtypes which don't cross-protect. Type O is the most FMDV showing variations, followed by type A. The other serotypes are greatly stable and do not have many subtypes.

FMD virus serotypes are currently differentiated by ELISA at the World Reference Laboratory, Pirbright, UK, and elsewhere following the methods developed by Abu Elzein (1979) and Crowther and Abu Elzein (1979). However, subtyping is usually achieved by the combination of serological and molecular biological techniques (Reweymamu 1978; Abu Elzein and Crowther 1982; Robson et al. 1979).

FMD virus has four major polypeptides known as virus protein 1 (VP1), VP2, VP3 and VP4. VP1 is the most significant as far as immunization and attachment to host cell is concerned. It is also responsible for the type and subtype specificity of the virus (Strohmair et al. 1982; Rowlands et al. 1971).

Isoelectric focusing (IEF) has been used with success in the study of virus polypeptides (King and Newman 1980; Glass and Millar 1994) and also to differentiate between closely related virus isolates (King et al. 1981; womey et al.1995).

The IEF technique is based on the separation of proteins according to their isoelectric point. Thus, the protein subunits that make up biological structures (e.g. viral structural proteins) can be separated in individual polypeptides with very high resolution (King et al. 1983; Twomey et al. 1995).

Foot-and-mouth disease (FMD) virus type '0' is the most endemic aphthovirus serotype in the Sudan. outbreaks due to this virus are experienced every now and then in the country (Abu Elzein 1983). The Sudanese isolates of FMD type '0' were previously subtyped using conventional serological tests (Abu Elzein and Newman 1982), and by competition ELISA (Abu Elzein and Crowther 1980).

In the present study, we compare some Sudanese type '0' FMD viruses with other standard type '0' vaccine viruses, in isoelectrofocusing studies. Results are correlated with previous serological and epidemiological data.

Materials and Methods

Type 'O' FMD viruses:

Type '0' FMD viruses used in this study: all of them were kindly supplied by the World Reference Laboratory (WRL), Pirbright, UK except KEN 120/64 which was kindly supplied by Dr. Mark Reyemamu, Welcome FMD Vaccine Laboratory , Pirbright, UK : '0' Sudan 3/1970 (D SUD 3/70) : was isolated from an FMD outbreak in Soba Fattening Scheme -Khartoum Province, in 1970.

'0' Sudan 1/1976 (D SUD 1/76) : was isolated from an FMD outbreak from El Gadarif district -Kassala province, in 1976.

'0' Sudan 1/1980 (0 SUD 1/80) : was isolated from an FMD outbreak from Kadaro quarantine, Khartoum, in 1980.

'0' /UK 1860/1967 (0, BFS) : this was the British field FMD virus strain which caused the 1967 outbreak in UK. It was isolated from Wrexham.

'0' Kenya 120/1964 (0 KEN 120/64) : this was a vaccine strain used in Kenya. It was kindly supplied by Dr. Mark Reyemanu, Welcome, FMD Lab. Pirbright, UK.

Virus growth and purification :

Viruses were grown in baby hamster kidney (BHK), clone 21, cell monolayers in Roux flasks at 37°C over night before freezing. To the hawed 1ysate, an equal volume of saturated ammonium sulpathe, pH 7.6 at 4°C was added. After centrifugation for 30 minutes at 5000 xg, the pellet was suspended in 0.04M Nap04, pH 7.6-0.1 M Nacl and clarified by centrifugation at 20000 rpm for 15 rpm for 15 min. in Beckman SW 27 rotor at 20°C. The Virus was pelleted by centrifugation at 2700 rpm for 2 hr and resuspended by sonication in 0.04 M NaP04 pH 7.5-0.1 M Nacl. Insoluble matter was removed by centrifugation at 1500 xg, and 0.1 volume of 10% sodium dodecyl sulphate (SDS) was added to the supernatant before layering on a linear 15 to 45% sucrose gradient in the same solvent and centrifuged tied for 70 min at 41.000 rpm, at 20°C, in an SW 41 rotor. The gradient was fractionated, and the virus was assayed by absorbency at 259 nm assuming a specific absorbance of 10 for a virus solution containing 1 mg of protein per m1 (Bachrach et al. 1964). Virus was then concentrated, by pelleting at 50.000 rpm in an SW 50.1 rotor for 1 hr at 20°C and was resuspended in 2 ml of PBS pH 7.4.

Electrofocusing :

The methods of King and Newman (1980) and Twomey et al. (1995), based on those of '0' Farrell 1975) were followed, using 9.5 M urea, 4% acrylamide (recrystallized). All other chemicals, buffers, incubations and detailed methodology were as described by King and Newman (1980).

The 01BFS 1860 was taken as the reference virus in this study. Each virus preparation was loaded separately on top of a gel (King and Newman, 1980). Also each virus was mixed, separately, with the reference virus (OlBFS 1860) and were then loaded on top of separate gels.

Results

Figure 1 shows the polypeptide bands of the viruses used in this study. VP1, VP2 and VP3 are quite conspicuous, whereas VP4 did not appear (see discussion).

VP1 : SUD 1/76 and 01BFs 1860 VP1 focused at the same isoelectric point. The same could be seen for Ken 120/64 and SUD 3/70. VP1 of SUD 1/76 and SUD 3/70 had very close isoelectric points; whereas the isoelectric point in which VP1, of SUD 2/80, focused as widely different from that of the other Sudanese viruses.

The isoelectric point of VP1 of Ken 120/64 was slightly different from that of the reference virus and also from that of SUD 1/76 (which was very similar to the reference virus).

The isoelectric point of VP1 of SUD 2/80 focused away from those of all the tested viruses.

VP2 : All strains, in this study, had conserved VP2, except SUD 1/76 which had less than 1 charge shift towards the anode.

VP3: This was conserved for 01BFs and SUD 3/70. SUD 2/80 and Ken 120/64 showed conserved VP3 which was different from that of the rest of the viruses. VP3 of SUD 3/76 focused very near to those of 01FBs 1860 and SUD 3/70.

Discussion

The high sensitivity of the IEF has qualified its utilization in the detection of minor differences in FMD virus proteins. Identical FMD virus strains are readily detected, and any minor differences are magnified. In the light of this, the technique has been applied with confidence and great success in situations where incrimination of a specific FMD virus strain, as the causative agent of an outbreak, was disputed (King et al. 1981). Moreover, as the FMD virus strains are known to undergo continuous antigenic changes with high frequency, again the IEF technique was found ideal in the detection of such antigenic changes (King and Newman 1980;

1983; Lombard and Arrowsmith -cited by King et al. 1983), especially in a specific country or region (King et al. 1983; Robson et al. 1979; Myer et al. 1994).

Application of the IEF in the present study has clarified the relationship between the examined virus strains.

As VP1 of the FMD virus is known to be the most epidemiologically significant polypeptide, in the virus; where as VP4 has no such significance, our purpose in this study, was to focus VP1 as clearly as possible and to ignore VP4.

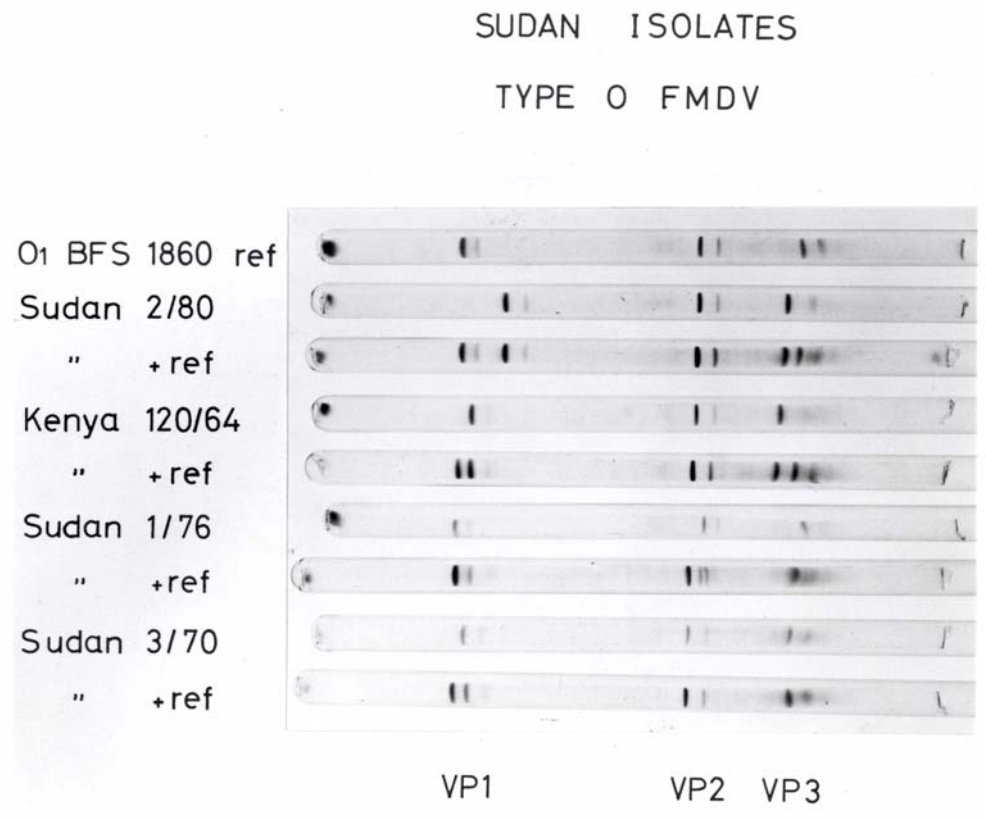
Results of the present study, showed that none of the examined strains were 100% identical. However, 01BSF, SUD 70, SUD 76 and Ken 120/64 were very close to each other and did not show wide shift in isoelectric points at the significant VP1. SUD 2/80 showed the widest variation from the rest of the viruses.

The previous serological and epidemiological data (Abu Elzein and Newman 1980; Abu Elzein and Crowther 1982; Abu Elzein 1983), showed that the same Sudanese type '0' isolates used in the present study, were closely related to 01BFS 1860 (R% value >70), except SUD 2/80 which was different from the rest of the Sudanese isolates and from the reference 01BFS (Abu Elzein 1988 unpublished data). This information seems to correlate well with that of the present electrofocusing results. This may lend support to the reports of King et al. (1983) who pointed out the potential of the IEF technique in virus epidemiology and those of Lombard and Arrow-Smith (as cited by King et al.1983), when their serological results were compared with those of the IEF for type '0' viruses (vaccine and field isolates). They showed that the Thai and Austrian isolates being clearly different from the others in both electrofocusing and serology. Also Myer et al. (1994) and Twomey et al. (1995) found similar results when they examined and compared field and laboratory FMD virus isolates using IEF.

We feel that IEF results together with highly sensitive serological tests such as competition ELISA (Abu Elzein and Crowther 1982; Anon 1998) would confirm differences between FMD virus strains, and will clarify situations of vaccine failures.

References

1. Abu Elzein E.M.E. (1979). The use of ELISA in foot-and-mouth diseases virus research. Ph.D. Thesis, Reading University .
2. Abu Elzein, E.M.E. (1983). Foot-and-mouth disease in the he Sudan. Rev. Sci.Tech. Off. Int. Epid. 2(1): 177-188.
3. Abu Elzein, E.M.E. and Crowther, J.R. (1982). Differentiation of foot-and-mouth disease virus strains using a competition ELISA. J. Virol. Meth. 3: 355-365.
4. Abu Elzein, E.M.E. and Newman, B.J. (1980). Subtyping of strains of FMD viruses type '0' in the Sudan 1970-1980. Bull. Off. Int. Epi., 92 (11-12): 1185-1191.
5. Anon (1998). International Atomic Energy Agency ManuaL Vienna on Immuno Assay Techniques.
6. Bachrach, H.L., Trautman, R. and Breese, Jr. (1964). Chemical and physical properties of virtually pure FMD virus. Am. J. Vet. Res. 25: 333-342.
7. Cooper, P.D., Agol, U.I., Bachrach, H.L. and Brown, F. (1978). Picornaviridae. Second Report. Intervirology, 10: 165-180.
8. Crowther, I.R. and Abu Elzein, E.M.E. (1979). Application of ELISA to the detection and identification of FMD viruses. I. Hyg. Camb., 83: 513.
9. Glass, E.I. and Millar, P. (1994). Induction of effective cross-reactive immunity by FMD peptides in critically dependent upon specific MHC peptide- T cell interactions. Immunology, 82: 1-8.
10. King, A.M.Q., McCahon, D., Newman, I.W., Crowther, I.R. and Carpenter, W .C. (1983). Electrofocusing structural and induced proteins of aphthovirus. In "Current topics in Microbiology and Immunology", 104: 219-232.
11. King, A.M.Q. and Newman, I.W. (1980). Temperature sensitive mutants ofMFD virus with altered structural polypeptides. I. Virol. 34 No.1: 59-66.
12. King, A.M.W., Underwood, B.O., McCahon, D., Newman, I.W. and Brown, F. (1981). Biochemical identification of viruses causing the 1981 outbreaks of FMD in the UK. Nature, 293, No.5832: 479-480.
13. Myer, R.F., Pacciarini, M. and Hilyard, E.I. (1994). Genetic variation of FMD virus from field outbreaks to laboratory isolations. Virus Res. 32: 299-312.
14. O'Farrell, P .H. (1975). High resolution two dimensional electrophoresis of proteins. I. BioI. Chem. 250: 4007-4021.
15. Reweymamu, M., Booth, I.C., Head, M. and Pay T.W.F. (1978). Microneutrali-zation tests for serological typing and subtying of FMD virus strains. I. Hyg. Camb. 81: 107-123.
16. Robson, K.I.H., Crowther, I.R., King A. and Brown, F. (1979). Comparative biochemical and serological analysis of five isolates of a single serotype of FMD virus. I. Gen. Virol. 45; 579-590.
17. Rowlands, D.I., sangar, D. and Brown, F. (1971). Relationship of the antigenic structure of foot-and-mouth disease virus the process of infection. I. Gen. Virol. 13: 85-93.
18. Strohmair, K., Franze, R., Adam, K.H. (1982). Location and characterization of the antigenic portion of the FMD immunizing protein. I. Gen. Virol. 59:295-306.
19. Twomey, T., France, L.L., Hassard, s., Burrage, T.G., Newman, I.F.E. and Brown, F. 1995. Characterization of acid resistant mutant of foot-and-mouth disease virus. Virology, 206; 69-75.



Legend to Figure 1.
Structural polypeptides (V PI, VP2, and VP3) of FMD diseases type '0' from Sudan as compared with the vaccine strains (01BFS and Ken 102/64) by isoelectric focusing.

مقارنة بعض عترات فيروس الحمى القلاعية النوع (و) بواسطة تقنية التوازن الكهربائي المتساوي

الطيب ابو الزين ، جون نيومان ، برندا نيومان

الملخص :

لقد تمت دراسة مقارنة لبعض عترات فيروس الحمى القلاعية النوع (و) بواسطة تقنية التوازن الكهربائي المتساوي، ابرزت النتائج توافق هذه التقنية مع التقنيات المصلية التي استعملت في هذه الدراسة سابقا. ناقشت الدراسة أهمية تقنية التوازن الكهربائي المتساوي في تصنيف الفيروسات.