

Communication

The isoelectrofocusing technique in comparison of some Sudanese type SAT-1 foot-and-mouth disease viruses

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ABU ELZEIN (E. M. E.), NEWMAN (J. W.), NEWMAN (B. J.).
Technique d'isoélectroconcentration dans l'étude comparée de quelques virus de fièvre aphteuse de type SAT-1 au Soudan. *Revue Élev. Méd. vét. Pays trop.*, 1989, 42 (3) : 327-329.

La technique d'isoélectroconcentration a été employée pour comparer les virus de la fièvre aphteuse de type SAT-1 au Soudan. Les résultats des tests ont été comparés aux données sérologiques et épidémiologiques obtenues précédemment sur les virus utilisés. Les possibilités d'utilisation du test, de même que les données sérologiques et épidémiologiques disponibles auparavant, sont discutées. *Mots clés* : Fièvre aphteuse - Virus - Méthode - Soudan.

Introduction

Foot-and-mouth disease is endemic in the Sudan (1, 9). The virus serotypes most endemic in the country are A, O and SAT-1 (1), whereas SAT-2 was recorded only once. The Sudanese FMD viruses isolated between 1967 and 1986 were subtyped by conventional serological methods (2, 3, 4, 5, 6) and by competition ELISA (3). However, no examination was made up to date, to compare their structural proteins in relation to the serological and epidemiological data.

FMD virus has four major structural polypeptides, known as virus protein I (VP₁, VP₂, VP₃ and VP₄), (8, 18). VP₁ is responsible for provoking type-specific antibodies (17). It is found on the surface of the virus and plays great role in virus attachment to host cells (16). Changes in the VP₁ lead to vaccine break down.

The other FMD virus proteins (VP₂, VP₃ and VP₄) are mostly group-specific and do not play major role in immunization.

Electrofocusing separates proteins according to their isoelectric point. The technique used in this study is

specifically concerned with electrofocusing in polyacrylamide gels containing 9.5 M urea. Under these conditions, the protein subunits that make up biological structures can be separated in individual polypeptides with very high resolution (9).

Electrofocusing has been applied in virology, in physical mapping of virus mutants ; in precursor-product relationship (e.g. mutations affecting mature aphthovirus polypeptides are always carried by precursors as well) ; in genetic recombination ; to determine molecular weights ; in strain identification ; in virus classification ; in peptide finger printing ; in secondary modification of protein (12) ; and in evolutionary changes.

Electrofocusing in FMD virus research has been utilized extensively by KING and co-workers all through the 1980's, and they pointed out the potential of the technique in virus epidemiology (12). The present study was intended to examine how SAT-1 virus isolates from Sudan compare in electrofocusing among themselves and with the standard vaccine strains used in Africa ; and how does this correlate with serological and epidemiological data.

The SAT-1 viruses

The SAT-1 viruses used in this study were Sudan 13/74 (SUD 13/74) ; Sudan 8/74 (SUD 8/74) ; Sudan 3/76 (SUD 3/76) ; Botswana 1/68 (BOT 1/68) ; Tanzania 155/71 (TAN 155/71). Their origin and description have been given earlier (6).

Virus growth and purification

Viruses were grown in baby hamster kidney (BHK), (Clone 21) cell monolayers in Roux bottles, at 37 °C over night before freezing. To the thawed lysate, was added an equal volume of saturated ammonium sulphate, pH 7.6 at 4 °C. After centrifugation for 30 minutes at 5,000 g, the pellet was suspended in 0.04 NaPO₄, -0.1 M NaCl, pH 7.6 and clarified by centrifugation at 70,000 g for 15 min in a Beckman SW27 rotor at 20 °C. The virus was pelleted by centrifugation at 130,000 g for 2 hours and resuspended by sonication in 0.04 M NaPO₄, -0.1 M NaCl, pH 7.5. Insoluble matter was removed by centrifugation at 1,500 g, and 0.1 volume of 10 per cent sodium dodecyl sulphate (SDS) was added to the supernatant before layering on a linear 15 to 45 per cent sucrose gradient in the same solvent and centrifugation for 70 min at 300,000 g, at 20 °C, in an SW41 rotor. The gradient was fractionated, and the virus was assayed by absorbance at 259 nm assuming (8). Virus was then concentrated, by pelleting at 300,000 g in an SW 50.1 rotor for one hour at 20 °C.

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Communication

Electrofocusing

The methods of KING and NEWMAN (11), based on those of O'FARRELL (16) were strictly followed ; using 9.5 M urea, 4 per cent acrylamide (recrystallized) and 0.2 per cent N,N'methylenebisacrylamide (recrystallized). All chemicals, buffers, incubations and detailed methodology were as described by KING and NEWMAN (11).

The Sudan 13/74 (SUD 13/74) virus was taken as « reference » in this study. Each virus preparation was loaded separately on top of a gel. Also, each virus was mixed separately with the reference virus (SUD 13/76) and were then loaded on top of separate gels.

Since the four FMD virus polypeptides cover such an extreme range of isoelectric points (11), it was not possible to electrofocus all of them together. Thus, the system was not allowed to reach equilibrium, but was stopped before VP₁ reached the bottom of the gel (this will be discussed later).

Results

Figure 1 illustrates the bands of SAT-1 FMD viruses used in the study. VP₁, VP₂ and VP₃ are quite conspicuous. VP₄ did not appear as it mostly remained at the origin during electrofocusing. The isoelectric point of each of the VPs of the reference virus, was conserved in the same position, in the five gels, in which the reference was included.

VP₁: The VP₁ of TAN 155/71 showed the widest variation from the reference virus and from the other

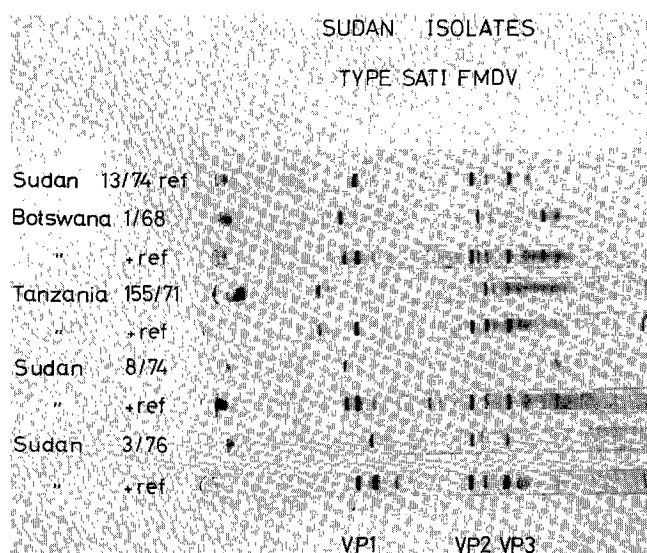


Fig. 1 : Isoelectric focusing bands of VP₁, VP₂ and VP₃ of Sudanese type SAT-1 FMD viruses as compared with the vaccine strains.

examined viruses. The Sudanese viruses did not show much variation for VP₁, although SUD 3/76 VP₁ was slightly different from that of the reference virus. BOT 1/68 VP₁ was evidently different from that of the reference.

VP₂: it was conserved in position for all viruses, although minor variation could be seen for BOT 1/68 and TAN 155/71 against the reference virus.

VP₃: it was conserved in position for all viruses tested, except for BOT 1/68 which showed wide variation from that of the reference virus.

Discussion

Up to now, the technique of electrofocusing has been little used for comparing viruses, although it provides a sensitive and rapid method for distinguishing closely related strains (12). Using electrofocusing, electrophoretic mutations affecting virus proteins have been detected in viruses collected at different times (13) and in conditional lethal mutants isolated in the laboratory (11, 14) and to identify viruses causing the 1981 outbreaks of FMD in Britain (12). In the Sudan SAT-1 viruses have only been isolated from diseased cattle, so far. Infection with this virus appears to take place in cycles, with a frequency range of 2-10 years. The latest outbreak, due to this virus, was recorded in 1976 (6), and the country remained free from SAT-1 infection for the last 13 years.

The reasons why recurrence of SAT-1 FMD virus infection, in the Sudan, is reflected in cycles, are not known nor maintenance of the virus in the environment have not been worked out either. However, earlier studies (7) have indicated presence of anti SAT-1 antibodies in sera from apparently healthy Sudanese goats. As natural clinical FMD was never seen in goats in the country, then goats may act as carrier of the virus ; and thus could maintain it in the environment.

In this study, great emphasis was made on the VP₁. This is because it is highly significant in immunization and classification of the virus into its types and subtypes. Loss of this protein through digestion with trypsin will render the remaining virus ineffective as far as vaccination is concerned.

Results of the electrofocusing indicate that both the African isolates (BOT 1/68 and TAN 155/71) were different in their VP₁ isoelectric points as compared with the Sudanese isolates. Again BOT 1/68 and TAN 155/71 showed difference in their isoelectric focusing points of the VP₁.

By correlating the isoelectric focusing results in this study with previous serological and epidemiological data (1, 3, 6), it could be shown that serology had

differentiated between BOT 1/68 and TAN 155/71 to the extent of regarding them as different subtypes.

Serological data on Sudanese SAT-1 viruses (2, 6) could also group the Sudanese SAT-1 isolates of (1970-1978) in one subtype, as they were closely related in R per cent values (> 70 per cent relationship). The serological data also showed that both BOT 1/68 and TAN 155/71 were widely different from the Sudanese isolates.

Results of the electrofocusing correlated well with the previous serological data, for the viruses in question. This result correlates with findings of KING *et al.* (12) when they compared the 1981 FMD type O, which caused the outbreaks in Britain, with the 1981 isolates in France, and with the O Lausanne strain which is used in the preparation of vaccines; and also with results of LOMBARD and ARROWSWITH, as cited by KING *et al.* (12), where they confirmed that the Thai and Austrian type « O » isolates being clearly different from the others according to both electrofocusing and complement fixation tests; and with that of ROBSON *et al.* (17) when they compared type « A » viruses by serology and chemical analysis and concluded that there was good correlation between the two.

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Isoelectric focusing technique (IEFT) was employed to compare type SAT-1 FMD virus from Sudan. Results of the IEFT tests were compared with available previous serological and epidemiological data on the viruses used. Possible potential uses of the test, in parallel with previously available serological and epidemiological data, are discussed. *Key words* : Foot-and-mouth disease - Virus - Technique - Sudan.

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