A serological study of Indian type O foot and mouth disease virus isolates

V.A. SRINIVASAN*, E.J. OULDRIDGE, M. HEAD and M.M. RWEYEMAMU**

Summary: Serological relationships between six type O FMD virus isolates from different parts of India were studied using two-dimensional microneutralisation tests. All the strains in the study were shown to be related to the O5 subtype. The vaccine strain, O India 53/79, exhibited the broadest serological spectrum, although small differences were observed with isolates from Northern and Western India. A recent isolate, O India 3/81, was found to have a narrow serological spectrum.

INTRODUCTION

The criteria for classification of foot and mouth disease virus (FMDV), particularly type and subtype differentiation have been reviewed by Brooksby (1968) and Pereira (1977). Type and subtype differentiation were based respectively on complete lack of or partial cross protection. Serological tests were used as an alternative to cross protection tests and the complement fixation test formed the basis for classification. Rweyemamu, Pay and Parker (1977) proposed that results of virus neutralisation comparisons should be preferred to those obtained in the complement fixation test, and reference antisera prepared from those strains which are in current vaccine production should be used for comparison of field strains. In the recent meeting of the International Association of Biological Standardization, 1981, it was proposed that the reference in vitro test system for serological differentiation of FMDV strains should be based on the virus neutralisation reaction and that the statistical significance of ‘r’ values should be taken into consideration (Anon., 1981).

Variation among type O viruses has been reported by several workers (Arrowsmith, 1977; Ouldridge et al., 1982). The present study was undertaken to compare the antigenic relationships among Indian type O FMDV isolates, originating from different geographical areas during various years, with a view to estimating the likely performance of the candidate vaccine strain against field strains.

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MATERIALS AND METHODS

Viruses.

Six type O FMD virus isolates were supplied by the World Reference Laboratory for FMD, Animal Virus Research Institute (AVRI), Pirbright. The strains were typed by the World Reference Laboratory, and received at the Wellcome FMD Laboratory, Pirbright as calf thyroid (Bty) or calf kidney (CK) or BHK adapted viruses. These strains were adapted to growth in BHK 21 monolayer cells. The details of the isolates as well as their passage history are furnished in Table I. Samples received by the World Reference Laboratory were designated by year of isolation and the number of isolates received in that year. Hence India 1/80 was the first sample received from India in 1980.

<table>
<thead>
<tr>
<th>Virus isolate</th>
<th>Place of origin</th>
<th>Type of animal</th>
<th>Passage history</th>
</tr>
</thead>
<tbody>
<tr>
<td>O India 53/79*</td>
<td>Nilgiris, Tamil Nadu, South India</td>
<td>Cattle</td>
<td>BHK 2 E/T+ BHK 8 Susp 4 BHK 1</td>
</tr>
<tr>
<td>O India 3/81**</td>
<td>Hyderabad, Andhra Pradesh, South India</td>
<td>Buffalo</td>
<td>CK 2 BHK 1 E/T BHK 6</td>
</tr>
<tr>
<td>O India 1/62***</td>
<td>Moradabad, Uttar Pradesh, North India</td>
<td>Cattle</td>
<td>BHK 7</td>
</tr>
<tr>
<td>O India 66/79*</td>
<td>Mathura, Uttar Pradesh, North India</td>
<td>Cattle</td>
<td>BHK 2 E/T BHK 6</td>
</tr>
<tr>
<td>O India 58/79**</td>
<td>Coimbatore, Tamil Nadu, South India</td>
<td>Cattle</td>
<td>Bty 1 E/T BHK 5</td>
</tr>
<tr>
<td>O India 1/80**</td>
<td>Kaira-Gujarat, North-western part of India</td>
<td>Cattle</td>
<td>CK 2 E/T BHK 5</td>
</tr>
</tbody>
</table>

* Ex. ICAR Regional Typing Centre Ranipet and Mathura.
** Isolated by NDDB staff.
*** Ex. AVRI.
+ E/T = treatment of cell culture harvest with ether.
Antisera.

Antisera were prepared as described by Rweyemamu, Booth and Pay (1977).

i) Guinea pig antisera.

For each virus 10 guinea pigs were inoculated intramuscularly with AEI-inactivated 140S antigen purified by the method of Brown and Cartwright (1963) which had been emulsified in Freund's incomplete adjuvant. The guinea pigs were re-inoculated 28 days later with a similar dose of freshly prepared antigen; the animals were exsanguinated 10 days later and the separated individual sera were pooled in approximately equal proportions, aliquoted and stored at −20°C until use.

The guinea pig antiserum against O5 India 1/62 was supplied by Miss A.E.M. Arrowsmith of the AVRI.

ii) Rabbit sera.

Rabbit sera were prepared by inoculation of groups of 4 rabbits with live tissue culture adapted virus and either exsanguinated at 28 days or re-inoculated after 28 days and exsanguinated at 35 days. Sera were pooled and stored as described above.

iii) Bovine vaccinate sera.

A group of four steers were vaccinated with monovalent type O (O India 53/79) vaccine and they were bled 21 days post vaccination. The sera were pooled before use.

Neutralisation tests.

The two-dimensional microneutralisation test was carried out as described by Rweyemamu et al. (1978). The mean titres of two or more replicate tests were used for the calculation of ‘r’ values, defined as the ratio in serum titre to heterologous and homologous viruses.

The significance of ‘r’ was determined by using the ‘t’ test. In this study, an estimate of pooled variance of 0.106 previously determined for the test was used rather than the individual variance for each comparison.

RESULTS

The ‘r’ values obtained in various tests with O India viruses are shown in Table II.

a) Relationship between O India 53/79 and other field strains.

Using serum from cattle after vaccination with O India 53/79, no significant differences between the isolates could be demonstrated (‘r’ values ranging from 0.44 to >1.00 with p>0.1).
### TABLE II

**Relationships between *O India 53/79* vaccine strain and other Indian virus isolates**

<table>
<thead>
<tr>
<th>Serum</th>
<th><em>O India 53/79</em></th>
<th><em>O India 3/81</em></th>
<th><em>O India 1/62</em></th>
<th><em>O India 66/79</em></th>
<th><em>O India 1/80</em></th>
<th><em>O India 58/79</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig serum</td>
<td>1.00</td>
<td>0.55</td>
<td>0.50</td>
<td>0.17**</td>
<td>0.21*</td>
<td>0.41</td>
</tr>
<tr>
<td>Rabbit convalescent serum</td>
<td>1.00</td>
<td>0.42</td>
<td>0.64</td>
<td>0.25*</td>
<td>0.25*</td>
<td>≥1.00</td>
</tr>
<tr>
<td>Bovine vaccinate serum</td>
<td>1.00</td>
<td>≥1.00</td>
<td>≥1.00</td>
<td>0.44</td>
<td>0.63</td>
<td>≥1.00</td>
</tr>
<tr>
<td>Guinea pig serum</td>
<td>0.10**</td>
<td>1.00</td>
<td>0.18**</td>
<td>0.15**</td>
<td>0.21*</td>
<td>0.13*</td>
</tr>
<tr>
<td>Rabbit convalescent serum</td>
<td>0.15**</td>
<td>1.00</td>
<td>0.44</td>
<td>0.20*</td>
<td>0.79</td>
<td>0.22*</td>
</tr>
<tr>
<td>Guinea pig serum</td>
<td>0.81</td>
<td>≥1.00</td>
<td>1.00</td>
<td>0.94</td>
<td>N.D.</td>
<td>≥1.00</td>
</tr>
<tr>
<td>Rabbit convalescent serum</td>
<td>0.46</td>
<td>0.12**</td>
<td>1.00</td>
<td>0.07**</td>
<td>0.26*</td>
<td>≥1.00</td>
</tr>
</tbody>
</table>

* Significantly different from 1.00 at *p* = 0.05
** Significantly different from 1.00 at *p* = 0.01

The strains *O India 3/81*, *O*<sub>2</sub> *India 1/62* and *O India 58/79* were shown to be related to *O India 53/79* when guinea pig and convalescent rabbit sera were used. However, the two viruses from northern and western parts of India, *O India 66/79* and *O India 1/80* showed a significant divergence from *O India 53/79*. The ‘r’ values for both viruses using convalescent rabbit sera were significantly different from 1.00 at *p* = 0.05 but not at *p* = 0.01. Similar results were obtained using guinea pig sera, except that the ‘r’ value for *O India 66/79* using guinea pig sera was found to be significantly different from 1.00 at *p* = 0.01.

b) **Relationship between *O India 3/81* and other strains.**

The guinea pig and convalescent rabbit sera raised against *O India 3/81* were used to determine the relationships between *O India 3/81* and other field isolates, including the *O India 53/79* vaccine strain. Sera to the recent isolate *O India 3/81* showed a narrow serological spectrum. Using guinea pig sera the relationship between *O India 3/81* and other Indian type *O* isolates was found to be significantly divergent. The ‘r’ value for *O India 1/80* was...
significantly different from 1.00 at $p=0.05$, while ‘r’ values for all other strains were significantly different from 1.00 at $p=0.01$.

With convalescent rabbit sera the ‘r’ value for O India 66/79 and O India 58/79 were significantly different from 1.00 at $p=0.05$, while the ‘r’ value for O India 53/79 was found to be significantly different from 1.00 at $p=0.01$.

c) Relationship between O$_5$ India 1/62 and other isolates.

All isolates examined were found to be related to O$_5$ India 1/62 using guinea pig sera.

Using convalescent rabbit sera, the ‘r’ values for O India 3/81 and O India 66/79 were shown to be significantly different from 1.00 at $p=0.01$, while the ‘r’ value for O India 1/80 was significantly different from 1.00 at $p=0.05$.

In summary, O India 53/79 showed the broadest serological spectrum and was found to be closely related to the O$_5$ subtype prototype strain India 1/62.

DISCUSSION

The study reported here was initiated to assess the serological appropriateness of strain O India 53/79 as a vaccine strain for India. This strain had already been shown to fulfil the other criteria laid down by Rweyemamu (1978) for a good vaccine strain, in that it grew readily in BHK suspension cells and was capable of producing potent vaccines with a long shelf life. In view of recent experiences of significant antigenic variation amongst type O viruses in Europe (Rweyemamu and Ouldridge, 1982$b$), the Middle East (AVRI WRL Information sheet No. 32), South East Asia (Ouldridge et al., 1982), and Kenya (Rweyemamu and Ouldridge, 1982$a$), it seemed appropriate to carry out a detailed analysis of the serological relationships among recent type O virus isolates from different parts of India.

The results of this study have shown that O India 53/79 has a broad serological spectrum, which favours its selection as the vaccine virus. Although some antigenic variation was observed, this strain would seem to be the most appropriate for the national campaign throughout India. In this study the bovine serum was found to be less discriminating than rabbit and guinea pig antisera emphasising the need for the use of a variety of antisera.

Although the viruses in this study could be regarded as belonging to the O$_5$ subtype, it is apparent that this group is not homogeneous since significantly divergent ($p<0.01$) relationships could be detected between isolated pairs within the group. This suggests that not all O$_5$ strains would be equally suitable as vaccine strains for use throughout India. In particular, the strain India 3/81 was found to have a narrow serological spectrum, illustrating that
neither membership of a particular subtype nor recent date of isolation *per se* constitutes the best criterion for selection of vaccine viruses. Narrow serological spectra and asymmetrical relationships have been observed before (Ouldridge *et al.*, 1982) and may be a more common feature of the evolution of new FMD strains than previously recognised. In order to minimise the development of new FMD strains in an endemic area, prophylactic campaigns should aim at maintaining a high level of effective herd immunity in order to provide protection against strains showing such antigenic divergence as found here. The use of a serologically appropriate strain in the vaccine is just one factor that needs to be considered. Other factors such as potency and method of application play a vital role (Rweyemamu, Pay and Simms, 1982).

**ACKNOWLEDGEMENT**

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ÉTUDE SÉROLOGIQUE DE SOUCHES DU VIRUS APHTEUX DE TYPE O ISOLÉES EN INDE. — V.A. Srinivasan, E.J. Ouldridge, M. Head et M.M. Rweyemamu.

Résumé : Les relations sérologiques entre six souches du virus aphteux de type O isolées dans différentes régions de l’Inde ont été étudiées par la microméthode de séroneutralisation bidimensionnelle.

Il ressort de cette étude que les souches étaient toutes apparentées au sous-type O.

ESTUDIO SEROLÓGICO DE CEPAS DEL VIRUS AFTOSO DE TIPO O AISLADAS EN INDIA. — V.A. Srinivasan, E.J. Ouldridge, M. Head y M.M. Rweyemamu.

Resumen : Se estudiaron mediante micrométodo de seroneutralización bidimensional, las relaciones serológicas entre seis cepas del virus aftoso de tipo O aisladas en distintas regiones de la India.

Del estudio se infiere que todas las cepas estaban emparentadas al subtipo O. La cepa vacunal, O India 53/79, presentaba el más amplio espectro serológico. No obstante, se observaron pequeñas diferencias con las cepas aisladas en la India del norte y del oeste. Se comprobó que una cepa recientemente aislada, O India 3/81, tenía un espectro serológico estrecho.
REFERENCES


