Studies with recent type O FMD isolates from South-East Asia

by

E.J. OULDRIDGE, M. HEAD, H. BUCK and M. M. RWEYEMAMU(*)

INTRODUCTION

The predominating FMD type in South-East Asia has in recent years been type O (Arrowsmith, 1977), although type A FMD was responsible for an important outbreak in the Philippines in 1975 (Charutamra, 1979) and type Asia 1 may be of increasing importance (World Reference Laboratory cumulative report 1980). Previous studies have shown that type O isolates from this area, as recently as 1978, demonstrated reasonably close relationships with O1 BFS 1860 (Rweyemamu et al., 1979). This strain appeared to be the most appropriate type O vaccine virus examined (Arrowsmith, 1977; Rweyemamu et al., 1979) and has been used successfully to eradicate FMD from Indonesia, the last recorded outbreak there being in 1979 (Anon., 1979; 1981). However, type O infection appears to be causing increasing concern in Thailand and Malaysia. In 1978, an outbreak, which occurred in Burma and central Thailand, spread into Southern Thailand and later to peninsular Malaysia. This outbreak appeared to be under control by late 1978 and few outbreaks of type O infection were recorded in 1979. In May 1980, however, a new outbreak of type O occurred in the central and eastern regions of Thailand. The disease, apparently originating in cattle, perhaps from the Thai-Kampuchean border area, spread into the intensive pig rearing areas around and to the south of Bangkok.

Despite the availability of three imported vaccines (of the O1 subtype) and vaccine produced by the Thai Livestock Department, the disease incidence grew worse over the succeeding months causing widespread morbidity and mortality especially in young pigs. There was disagreement as to the efficacy of the four vaccines used to control the outbreak. A cross challenge test

(*) Wellcome Foundation Ltd., Wellcome Foot-and-Mouth Disease Vaccine Laboratory, Ash Road, Pirbright, Woking, Surrey GU24 0NQ.
showed that pigs vaccinated only once with European O1 vaccines were apparently less well protected against the Thai field virus than could be expected from the high potency of the vaccines used. Pigs that had been vaccinated twice with these vaccines withstood infection with the Thai virus. From this limited experience the Thai authorities recommended the use of O1 vaccines by a primary course of two injections. However, reports from the field suggested that where O1 BFS 1860 oil vaccine had been used either under a single or double vaccination regimen, infection was successfully contained in piggeries.

In July 1980, an outbreak in South Thailand (Surat Thani) was recorded, perhaps the consequence of the illegal movement of infected pigs. This infection was contained by slaughter. At about the same time disease reappeared on the Southern Thailand/Malaysian border and had spread into Malaysia by September 1980. This outbreak appeared to be unrelated to the Central Thailand outbreak.

In Malaysia, the rigorous vaccination and slaughter enforced after the 1978 outbreak appeared to have eliminated infection and during 1980 it was relaxed, so that by September 1980, many cattle along the Thai-Malaysian border had either never been vaccinated or vaccinated at least one year previously. This probably contributed to the initial spread of disease through Northern Malaysia. By October 1980, FMD had been recorded in both cattle and pigs. Although isolated outbreaks were recorded as far south as Kuala Lumpur and Malacca, these were quickly controlled by slaughter. Extensive vaccination with monovalent O1 vaccines and a quarantine scheme appear to be bringing the disease under control in the four Malaysian Northern States. Further evidence for the efficacy of O1 vaccines in Malaysia was provided by a cross challenge test carried out by the Malaysian veterinary authorities. They reported that cattle vaccinated with O1 BFS 1860 withstood challenge with the Malaysian 1980 field virus isolate.

The conflicting evidence from Thailand and Malaysia, and the persistence of infection in Central Thailand during months usually associated with reduced levels of disease led us to undertake a serological evaluation of the Thai and Malaysian type O viruses in order to assess their antigenic interrelationships and their relation to European O1 vaccine viruses.

**MATERIALS**

**VIRUSES**

1) O1 BFS 1860 European O1 vaccine strain.
2) O Thailand 1/80 : candidate vaccine strain.
3) O1 Lausanne 65 European O1 strain.
4) Isolated before 1980 outbreaks : O Burma 11/77, O Burma 11/78, O Malaysia 1/78, O Hong Kong 15/71, O Hong Kong 17/22, O Hong Kong 8/77 and O Hong Kong 18/77.
5) Isolated during 1980: O Thailand 1/80: isolated from pigs in the Bangkok area; O Malaysia 1/80: isolated from cattle in the Northern Peninsular Malaysia.


All field strains were adapted to BHK monolayer growth. All virus strains originated from the World Reference Laboratory for FMD at the AVRI, Pirbright.

**ANTISERA**

i) To O1 BFS 1860.

AS 199  Sera taken from convalescent cattle (BCS).
AS 323  
AS 359  Pooled sera taken 4-5 weeks after vaccination from groups of guinea pigs vaccinated with inactivated, purified 140S antigen emulsified in complete Freund's adjuvant (GPS).
AS 377  
AS 533  
AS 869  
AS 666  Pooled cattle sera taken 3 weeks after vaccination with Wellcome monovalent, aqueous O1 BFS 1860 vaccine (BVS).
AS 667  
AS 594  

ii) To O1 Lausanne 65.

AS 652  Serum taken from a convalescent steer (BCS).

iii) To O Thailand 1/80.

AS 571  Pooled rabbit serum taken 28 days after inoculation with live BHK adapted virus (RCS).

AS 956  Pooled cattle sera taken 2 weeks after revaccination with Wellcome monovalent aqueous O Thailand 1/80 vaccine (BVS).

**METHODS**

a) Serological Comparisons.

The serological comparison of virus strains was made using the two-dimensional microneutralisation test carried out in matched pairs (Rweyeremamu et al., 1978).

b) Potency Evaluation of Vaccines.

Pirbright vaccine batch 370 (O1 BFS 1860) was inoculated at a 1/2 dilution into eight steers by the subcutaneous route at a dose volume of 3 ml per steer. The animals were bled 21 days later, and the sera tested in the metabolic inhibition test (Martin and Chapman, 1961) and the two-dimensional microneutralisation test described above.
RESULTS

Table 1 shows serological comparisons made between O1 BFS 1860 and field virus strains isolated as recently as 1978. Isolates from Burma, Malaysia and Hong Kong all show close relationships with O1 BFS 1860 (r values ranging from 0.36 to 0.98 and none significantly different from 1.00 at p = 0.05).

Table 2 shows serological comparisons of 1980 isolates from Thailand and Malaysia with O1 BFS 1860 and O1 Lausanne. O Thailand 1/80, which was isolated in the Bangkok area, shows an asymmetric relationship with both O1 BFS 1860 and O1 Lausanne. The relationships given by O Thailand 1/80 sera with either O1 strain (r = 0.01) are highly significantly different from r = 1.00 (p<0.01). The relationships given by sera to the O1 strains are greater (r = 0.21-0.25) but still significantly divergent from r = 1.00 at p = 0.05.

O Malaysia 1/80 virus appeared closely related to the O1 BFS 1860 vaccine virus (r>=0.98). The relationship is similar to that demonstrated for O Malaysia 1/78 (see Table 1) suggesting that the 1980 Malaysia outbreak probably represented a flare up of the same infection along the Thailand-Malaysian border rather than a fresh introduction of the virus from Central Thailand. To date all the type O virus isolates from Malaysia between 1978 and July 1981 have maintained this close relationship with O1 BFS 1860 vaccine virus (Table 2).

In order to assess the likely efficacy of O1 vaccines against the O Thai virus, the potency against O Thailand 1/80 of one batch of O1 BFS 1860 vaccine (Batch 370) was examined in our laboratory. Table 3 shows the log antibody titres for the eight cattle vaccinated determined by both metabolic inhibition and two-dimensional microneutralisation tests. The results show that the vaccine was potent against the homologous virus, but the heterologous response against O Thailand 1/80 virus was low. This suggested that the efficacy of O1 BFS 1860 vaccines of similar potency would be suboptimal in primo-vaccinated cattle. However, it could be expected from the titres against O Thailand 1/80 virus that a secondary vaccination would provoke a booster response that would be of sufficient magnitude to limit the spread of infection and confer an adequate protection against O Thailand 1/80 under field conditions.

DISCUSSION

The results presented here, together with the epidemiological evidence, suggest that a new strain of type O virus arose in Central Thailand either in late 1979 or early 1980. This virus appears to be confined to Central Thailand at present. Outbreaks in peninsular Thailand and Malaysia appear to be adequately controlled by present measures and may be unconnected with the Central Thailand outbreak.
TABLE 1: Relationships between type O1 BFS 1860 virus and South-East Asian isolates until 1978.

Table of r values determined by two-dimensional microneutralisation.

<table>
<thead>
<tr>
<th>Field strain</th>
<th>Serum to: O1 BFS 1860</th>
<th>Serum to: O Burma 11/77</th>
<th>Serum to: O Burma 11/78</th>
<th>Serum to: O Malaysia 1/78</th>
<th>Serum to: O Hong Kong 15/71</th>
<th>Serum to: O Hong Kong 17/22</th>
<th>Serum to: O Hong Kong 8/77</th>
<th>Serum to: O Hong Kong 18/77</th>
</tr>
</thead>
<tbody>
<tr>
<td>O1 BFS 1860</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS 199 BCS</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.78</td>
<td>0.83</td>
<td>0.60</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>AS 359 GPS</td>
<td>ND</td>
<td>ND</td>
<td>0.71</td>
<td>0.36</td>
<td>0.74</td>
<td>0.52</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>AS 377 GPS</td>
<td>ND</td>
<td>0.85</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>AS 667 BVS</td>
<td>0.66</td>
<td>0.96</td>
<td>0.98</td>
<td>ND</td>
<td>ND</td>
<td>0.48</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>AS 869 GPS</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.6</td>
<td>0.52</td>
</tr>
</tbody>
</table>
### TABLE 2: Relationships between European type O₁ vaccine strain and South-East Asian strains isolated in 1980/1981.

Table of r values determined by two-dimensional microneutralisation.

<table>
<thead>
<tr>
<th>Serum to:</th>
<th>O₁ BFS 1860</th>
<th>O Lausanne 65</th>
<th>O Thailand 1/80</th>
<th>O Malaysia 1/80</th>
<th>O Malaysia 9/81</th>
<th>O Malaysia 17/81</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₁ BFS 1860</td>
<td>1.0</td>
<td>ND</td>
<td>0.21*</td>
<td></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AS 199 BCS</td>
<td>1.0</td>
<td>ND</td>
<td>0.29*</td>
<td>0.98</td>
<td>0.45</td>
<td>ND</td>
</tr>
<tr>
<td>AS 323 BCS</td>
<td>1.0</td>
<td>ND</td>
<td>0.23*</td>
<td>0.98</td>
<td>0.45</td>
<td>ND</td>
</tr>
<tr>
<td>AS 533 GPS</td>
<td>1.0</td>
<td>ND</td>
<td>0.25*</td>
<td>0.98</td>
<td>0.45</td>
<td>ND</td>
</tr>
<tr>
<td>AS 666 BVS</td>
<td>1.0</td>
<td>ND</td>
<td>0.25*</td>
<td>0.98</td>
<td>0.45</td>
<td>ND</td>
</tr>
<tr>
<td>AS 594 BVS</td>
<td>1.0</td>
<td>ND</td>
<td>0.25*</td>
<td>0.98</td>
<td>0.45</td>
<td>ND</td>
</tr>
<tr>
<td>O₁ Lausanne 65</td>
<td>ND</td>
<td>1.0</td>
<td>0.25*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AS 652 BCS</td>
<td>ND</td>
<td>1.0</td>
<td>0.25*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>O Thailand 1/80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS 571 RCS</td>
<td>0.01*</td>
<td>0.01*</td>
<td>1.0</td>
<td>0.01*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AS 956 BVS</td>
<td>0.01*</td>
<td>ND</td>
<td>1.0</td>
<td>0.05*</td>
<td>0.12</td>
<td>0.07</td>
</tr>
</tbody>
</table>

ND = Not done
BCS = Convalescent bovine serum
GPS = Guinea pig serum
BVS = Vaccinated bovine serum
RCS = Convalescent rabbit serum
* = Results significantly different from 1 at p = 0.05
<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Serum Assay Method</th>
<th>Antibody Titre Log SN 50 against:</th>
<th>Mean (S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O Thailand 1/80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O1 BFS 1860</td>
<td></td>
</tr>
<tr>
<td>NK 91</td>
<td>2.35*</td>
<td>&lt; 1.39***</td>
<td>1.42**</td>
</tr>
<tr>
<td>NK 92</td>
<td>2.46</td>
<td>&lt; 1.09</td>
<td>1.03</td>
</tr>
<tr>
<td>NK 93</td>
<td>2.84</td>
<td>1.24</td>
<td>1.42**</td>
</tr>
<tr>
<td>NK 94</td>
<td>2.95</td>
<td>1.24</td>
<td>1.03</td>
</tr>
<tr>
<td>NK 95</td>
<td>2.76</td>
<td>1.24</td>
<td>1.42**</td>
</tr>
<tr>
<td>NK 96</td>
<td>2.35</td>
<td>1.24</td>
<td>1.03</td>
</tr>
<tr>
<td>NK 97</td>
<td></td>
<td></td>
<td>1.42**</td>
</tr>
<tr>
<td>NK 98</td>
<td></td>
<td></td>
<td>1.03</td>
</tr>
</tbody>
</table>

**Serum Assay Method**
- Colour Test
- 2-D Microneutralisation Test

**Mean (S.D)**
- Mean four tests
- Mean two tests
- Single test results
The O1 vaccine would probably be efficacious in controlling disease throughout Thailand provided it is used as part of a regular prophylactic campaign. Alternatively, to ensure protection against the new strain, a bivalent vaccine composed of O Thailand 1/80 and O1 BFS 1860 may be recommended for use in Thailand. O Thailand 1/80, alone, would not appear to give satisfactory cover throughout Thailand and Malaysia.

The manner in which the new strain arose has interesting implications. This outbreak, which had been smouldering in Eastern and Central Thailand, only seemed to become of major importance after its appearance in the pig population. The rapid spread of disease in pigs could have been due partly to the intensive conditions in which they are kept in this area and to the generally low level of vaccination prior to this epidemic. It also seems likely that the antigenic differences reported here were a contributory factor. The antigenic change may have resulted when the focus of infection moved from cattle to pigs. Some changes in antigenic character have been observed when strains are adapted to alternative replicative systems (Cowan et al., 1974; Parry et al., 1978). Alternatively, new strains may occur in endemically infected areas after passage in animals with sub-optimal levels of immunity (Hyslop and Fagg, 1965). It could be that the antibody in these animals encourages the emergence of variant strains. This effect has been observed for FMD under controlled conditions in partially immunised cattle (Hyslop and Fagg, 1965).

The serological relationship between O1 viruses and the O Thailand 1/80 strain appears to be asymmetric in that r values obtained using O Thailand 1/80 sera against O1 strains are much lower than r values obtained using sera to O1 strains against O Thailand 1/80. Similar asymmetric relationships have been reported by Arrowsmith (1977) and have been observed repeatedly in our laboratory. For example, serum to the Colombian vaccine strain O Colombia 7250, gives r values which are not statistically different from r = 1 at p = 0.05 (range 0.4-1.00) with four recent type O Colombian field isolates, whereas sera to the recent isolates give r values which are significantly divergent from 1.00 at p = 0.05 (range 0.06-0.19) (unpublished data). It is possible that asymmetric relationships with vaccine viruses may be typical of strains which emerge as a consequence of antigenic drift in endemic areas. In a recent paper by Domingo, Davila and Ortin (1980), where a number of FMD strains from outbreaks in an endemic area were compared by T1 oligonucleotide mapping, it was demonstrated that FMD isolates from the same outbreak were genetically variable, differences even being observed between two samples of one isolate. It was suggested that this genetic variability could be linked to antigenic variation. If populations of FMDV in an endemic area were antigenically heterogeneous, then the presence of sub-optimal levels of antibody to predominantly one group of variants would encourage the proliferation of others. So samples of the original population may contain all the variants represented in the second but not vice versa. This would lead to an apparent asymmetry of relationships between the two populations. Over a period of time, new spontaneous mutations within the second population and further selection against established variants lead to the generation
of new subtypes. Such a theory of subtype development requires that subtypes should be overlapping rather than discrete, and this has been observed by Arrowsmith (1975) in a study of Middle Eastern type A viruses.

The generation of new subtypes by antigenic drift has important implications for the selection and use of vaccine strains. Tactical utilisation of vaccines to maintain a high level of effective herd immunity and thereby limit the spread of virus in animals with sub-optimal levels of antibody would be advisable when antigenic variants are suspected. When new strains arise that demonstrate the asymmetric relationships discussed here, extensive prophylactic vaccination of the area locally affected with the existing vaccine strain may be sufficient to prevent the spread of infection. Inclusion of the new strain in the vaccine programme may be limited to a short term local application only, with reversion to the regular vaccine strain as soon as the spread of the disease in the field is contained.

ACKNOWLEDGMENTS

We are grateful to Dr. K.J. O'Reilly and the staff of the Serum Assay Unit for titration of cattle sera in the cell metabolic inhibition test, to Mr. P. Hingley for statistical advice and to Mrs. J. Greenwood and Mrs. F. Purse for technical assistance.

SUMMARY

The epidemiology of type O FMD in Thailand and Malaysia during 1978 to 1980 is discussed. Isolates from South-East Asia from 1977 to 1980 are compared with the O1 BFS 1860 vaccine strain using two-dimensional microneutralisation tests. Only one strain, O Thailand 1/80 was shown to differ significantly from the O1 virus strains BFS 1860 and Lausanne 65. The serological relationship between O Thailand 1/80 and the O1 strains was shown to be asymmetric. The efficacy of O1 vaccines for controlling O Thailand 1/80 was assessed in neutralisation tests, and the use of O1 vaccines in the context of a regular prophylactic campaign was recommended, with perhaps the local use of O Thailand 1/80 vaccines if necessary. The possible mechanisms of antigenic drift and their relevance to vaccination are discussed.

REFERENCES


