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49 **Abstract**

50 Foot-and-mouth disease (FMD) virus exists as 7 serotypes within which are numerous  
51 variants necessitating careful selection of vaccine strains. Currently, a serological  
52 assay system based on the use of polyclonal vaccine antisera is widely used for this  
53 selection. However, inherent variability in the matching antisera used makes the tests  
54 poorly reproducible and difficult to interpret. In this study, we have explored the  
55 possibility of replacing or supplementing the polyclonal antibody (PAb) based method  
56 with one based on use of monoclonal antibodies (MAb). Panels of MAbs raised  
57 against two serotype O vaccine strains were examined for reactivity with 22 field  
58 viruses, isolated over a ten year period between 1991 and 2001. Antigenic site 2 was  
59 found to comprise more than one epitope. The sequence variation in capsid protein  
60 VP2 harbouring antigenic site 2 was analysed and the amino acid residues at positions  
61 79 and 134 appeared to greatly influence the binding of site 2 MAbs. Prediction of  
62 antigenic match based on MAb reactivity did not correlate closely with the results of a  
63 PAb-based “gold-standard” method and it was concluded that a wider panel of MAbs  
64 are needed that recognise all protective epitopes present on the surface of FMD virus  
65 together with a better understanding of those epitopes which are important in  
66 conferring protection.

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**82 Introduction**

83 Foot-and-mouth disease (FMD) is a highly contagious and economically devastating  
84 disease of cloven-hoofed animals which can severely constrain international trade of  
85 animals and animal products. Some countries are currently recognised as being free of  
86 FMD; however, the disease is still endemic in many countries in Asia, Africa, the  
87 Middle East, South America and at the periphery of the European Union. FMD is  
88 caused by a virus in the genus *Aphthovirus* within the family *Picornaviridae*.  
89 Antigenically, this virus exists as 7 distinct serotypes and there are also subtypes  
90 within each serotype and following vaccination the response is only effective within  
91 its serotype although not against all subtypes (Brooksby, 1982; Cartwright et al.,  
92 1982; Mattion et al., 2004). In addition, new variant viruses are emerging  
93 periodically. Consequently, vaccine strain requirements differ according to the types  
94 and subtypes of virus prevailing globally and vaccines have to be selected with care,  
95 whether it be for prophylactic use in FMD-endemic countries or for incorporation into  
96 reserves for emergency use in FMD-free countries.

97 Methods of vaccine strain selection mainly rely on serological approaches (Paton et  
98 al., 2005). A commonly used method is to derive relationship values (“r” values)  
99 between FMD viruses using pools of antisera prepared against each vaccine strain to  
100 be matched. The antigenic similarities between vaccine strains and field isolates are  
101 estimated from their comparative reactivity with the appropriate serum pool using a  
102 neutralisation test (Rweyemamu et al., 1978) or an ELISA system (Kitching et al.,  
103 1988). Alternatively, the complement fixation test (CFT) can be used for this purpose  
104 (Alonso et al., 1987), although use of this test is now largely restricted to South  
105 American countries. There are various factors which may affect the results obtained  
106 from the in vitro matching tests. However, a major difficulty is the standardization of  
107 the polyclonal serum raised against the vaccine viruses, including both the sera

108 collected from vaccinated target species (usually cattle) and the antisera raised in  
109 rabbits and guinea-pigs for use as capture or detector antibodies in ELISA or the CFT.  
110 An approach to overcome this limitation to reproducibility encompasses the use of  
111 consistent and replenishable reagents such as monoclonal antibodies (MAb). This  
112 study explores the possibility of replacing the polyclonal antibody (PAb) based  
113 method with one based on a panel of MAbs and further more examines some of the  
114 key amino acid residues that assist the interactions between MAbs and epitopes.

115

#### 116 **Materials and Methods:**

117 **Cells and viruses:** Twenty-four serotype O viruses from Asia (n=18), Europe (n=5)  
118 and North Africa (n=1) were used in this study (Tab 1). Two were the vaccine strains  
119 O<sub>1</sub> Manisa and O<sub>1</sub> Lausanne that were originally isolated in Turkey and Switzerland,  
120 in 1969 and 1965 respectively. The other twenty-two viruses were isolated over a ten  
121 year period between 1991 and 2001. All were derived from bovine/porcine epithelial  
122 samples, submitted to the Food and Agriculture Organisation's World Reference  
123 Laboratory (WRL) for FMD at Pirbright, and were initially grown in primary bovine  
124 thyroid cells (BTY) with subsequent passage in either BHK-21 or IB-RS2 cells. All  
125 the viruses were grown to an equal passage level. Cell culture supernatant fluids  
126 containing virus were clarified by centrifugation at 2500 rpm for 15 min and then  
127 mixed with an equal volume of sterile glycerol and stored at -20°C until use.

128

129 **Polyclonal serum:** Rabbit and guinea-pig anti-FMDV polyclonal sera were prepared  
130 against purified serotype O FMD viruses (O<sub>1</sub> Lausanne and O<sub>1</sub> Manisa). These  
131 antisera are routinely used in WRL Pirbright for the detection of FMD viruses in an  
132 indirect sandwich ELISA. These antisera were raised in rabbits and guinea pigs by

133 immunising the animals with inactivated, purified 146S FMD virus particles in  
134 Freund's complete and/or incomplete adjuvant as described by Ferris and colleagues  
135 (Ferris et al., 2005). The antisera were mixed with an equal volume of normal bovine  
136 serum before use to absorb antibodies produced against bovine serum proteins  
137 associated with the purified virus inocula in order to prevent potential cross reactivity.  
138 21 day post-vaccination bovine antisera (bvs) used in the micro neutralisation assay  
139 were derived from cattle potency tests, carried out at Pirbright. For each antigen, a  
140 pool of sera from at least five animals was used in the serological tests.

141

142 **Monoclonal antibodies:** The monoclonal antibodies (MAbs) used in this study had  
143 been produced against the O<sub>1</sub> Lausanne or O<sub>1</sub> Manisa vaccine strains. The O<sub>1</sub>  
144 Lausanne panel comprised six well characterized neutralising MAbs against four  
145 antigenic sites (Crowther et al., 1993; Kitson et al., 1990) and the anti O<sub>1</sub> Manisa  
146 MAbs comprised seven well characterised neutralising MAbs directed against  
147 antigenic site 2 (Aktas and Samuel, 2000). The positions of the critical residues of  
148 these MAbs are presented in Table 2. In addition, there were also six non-neutralising  
149 O<sub>1</sub> Manisa MAbs (3H3, 3A4, 3G3, 4B12, 4C8, 4H6) provided by a commercial  
150 company (Bayer Healthcare, Germany). The epitopes recognised by these non-  
151 neutralising MAbs and position of the critical residues have not been determined.

152

153 **Antigenic profiling:** An antigen profiling ELISA, as described by Aktas and  
154 colleague (Aktas and Samuel, 2000) was used to determine the reactivity of individual  
155 field isolates against the MAbs. Briefly, cell culture grown viruses (pretitrated with  
156 type-specific guinea pig PAb to produce similar optical densities) were trapped by a  
157 type-specific rabbit PAb and reacted with each MAb and with a type-specific guinea

158 pig PAb. The reactivity was measured by incubation with rabbit anti-mouse or guinea  
 159 pig HRP conjugate and final colour development with ortho-phenylenediamine (OPD)  
 160 substrate. For reference, the vaccine virus, against which the MAbs were originally  
 161 raised was included in each test, as well as a blank (B) with no virus.

162 The reactivity of each MAb with a given field isolate (X) compared to the guinea pig  
 163 PAb was calculated by subtracting the respective blanks using the formula:  $R_X =$   
 164  $(OD_{MAbX} - OD_{MAbB}) / (OD_{PAbX} - OD_{PAbB})$ . These values were then related to those  
 165 obtained against the homologous reference virus (the virus to which the MAbs were  
 166 originally raised) by using the formula:  $\%reactivity = R_X / R_{Ref} \times 100$ . Values of 20%  
 167 or greater were considered positive.

168 In order to calculate the % antigenic homology of a particular field isolate to the  
 169 parent vaccine virus, the mathematical formula used by Garcíabarreno and colleagues  
 170 (Garcíabarreno et al., 1986) for African swine virus was used with slight  
 171 modifications. Briefly, the formula used was as follows:

$$172 \quad (0 \times a + 1 \times b + 2 \times c / 2 \times a+b+c) \times 100$$

173 where a is the number of MAbs showing 0-20% reactivity in a particular panel

174 where b is the number of MAbs showing 21-50% reactivity in a particular panel

175 where c is the number of MAbs showing 51-100% reactivity in a particular panel

176 and a+b+c is the total number of MAbs in a given panel.

177 0, 1 and 2 are the arbitrary weights given to MAbs in each reactivity class.

178

179 **Two-dimensional micro neutralisation assay:** A two-dimensional micro-  
 180 neutralisation test, similar to that described by Booth and colleagues (Booth et al.,  
 181 1978) was used. Briefly, doubling dilutions of antibody (bvs either against O1  
 182 Manisa or O1 Lausanne) were reacted with 0.5 log<sub>10</sub> dilutions of appropriate virus for

183 1hr at room temperature. BHK-21 or IB-RS2 cells were added as indicators of  
184 residual infectivity and the test incubated at 37°C for 3 days prior to fixing and  
185 staining. Antibody titres were calculated from regression data as the log<sub>10</sub> reciprocal  
186 antibody dilution required for 50% neutralisation of 100 tissue culture infective units  
187 of virus (log<sub>10</sub>SN<sub>50</sub>/100 TCID<sub>50</sub>). The antigenic relationship of viruses based on their  
188 neutralisation by antibodies is given by the ratio: 'r<sub>1</sub>' = neutralising antibody titre  
189 against the heterologous virus/neutralising antibody titre against the homologous  
190 virus. The significance of differences in the values of 'r<sub>1</sub>' obtained by the polyclonal  
191 antiserum was evaluated according to the criteria of Rweyemamu and Hingley  
192 (Rweyemamu and Hingley, 1984). According to these authors, serological  
193 relationships between two viruses in the range 'r<sub>1</sub>' = 0.3 - 1.0 are indicative of  
194 reasonable levels of cross protection whereas values less than 0.3 indicate very  
195 dissimilar strains and the need to acquire, or develop, another vaccine strain.

196 **Sequencing:** The nucleotide sequence of capsid protein VP2 of selected viruses was  
197 either retrieved from the FMDV sequence database available at Pirbright or  
198 determined by sequencing in order to compare their deduced amino-acid  
199 compositions. RNA extraction from the cell culture grown viruses was performed  
200 using standard techniques. RT-PCR was carried out using 'Ready to go RT-PCR  
201 beads' (Amersham Biosciences) as described by Knowles and colleagues (Knowles et  
202 al., 2005) using the primers FMD-L463F (ACCTCCRACGGTACGC) and O-1C361R  
203 (GGIGGGGCRTAIGCAAYCATGTA) which amplified the amino terminal half of  
204 the capsid-coding region. Prior to sequencing, PCR products were purified using the  
205 QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions.  
206 Cycle sequencing was carried out using the Beckman CEQ8000 DNA analysis  
207 system. Nucleotide sequences of the isolates were aligned using the CLUSTAL X

208 multiple sequence alignment program (Thompson et al., 1997) and the predicted  
209 amino acid sequences were translated using BioEdit 7.0.1 (Hall, 1999).

210 **Statistical analysis:** Statistical analyses were performed on antigen homology values  
211 obtained from a PAb based micro neutralisation test and MAb based ELISA using the  
212 Minitab release 12.21 software.

213

214 **Results:**

215 **Antigenic profiling:** The reactivity of O<sub>1</sub> Lausanne and O<sub>1</sub> Manisa MAbs with the  
216 different type O viruses were carried out using the antigenic profiling ELISA. All the  
217 tests were carried out at least twice and the mean values for O<sub>1</sub> Lausanne and O<sub>1</sub>  
218 Manisa panel are presented in Table 3 and 4, respectively. In case of O<sub>1</sub> Lausanne  
219 panel, the MAbs directed against site 1 (B2, D9) and site 5 (OC3) recognised all of  
220 the 24 viruses employed in this study. In contrast, the site 3 MAb (C8) reacted with  
221 only half of the isolates. The site 2 MAbs demonstrated two distinct reactivity  
222 patterns; C9 reacted with 21 viruses, whilst C6 reacted with only 11. The neutralising  
223 O<sub>1</sub> Manisa MAbs demonstrated various reactivity patterns against the 24 serotype O  
224 viruses (Table 3). The site 2 MAb, SA107 detected all of the viruses, whereas the  
225 other MAbs (also directed at antigenic site 2), exhibited two distinct reactivity  
226 patterns (Mabs SA120, SA113, SA127 and SA85 showed one pattern whereas Mabs  
227 SA88 and SA98 exhibited another pattern) and failed to recognise or recognised  
228 weakly nine viruses including O VIT 4/97 and O VIT 7/97 that were not recognised at  
229 all. Each of the six non-neutralising O<sub>1</sub> Manisa MAbs demonstrated a unique pattern  
230 of reactivity ranging from 3H3 that detected most of the viruses to 3G3 that only  
231 detected its parental strain.

232

233 Based on the reactivity of the MAbs against different field isolates, percentage  
234 antigenic homologies were calculated for each field isolate with respect to the  
235 appropriate vaccine strain, as described in the materials and methods, and the results  
236 are presented for vaccine strains O<sub>1</sub> Lausanne (Fig 1a) and O<sub>1</sub> Manisa (Fig 1b). It  
237 should be noted that procedurally all MAbs were given equal weighting within each  
238 panel although some may react with similar/identical epitopes to one another and in  
239 the case of other MAbs, the epitopes have not been defined. The antigenic relatedness  
240 of the type O field viruses to O<sub>1</sub> vaccine strains were found to be much higher in O<sub>1</sub>  
241 Lausanne panel (ranging between values 0.6 to 1.0) than those in O<sub>1</sub> Manisa panel  
242 (ranging between values 0.07 to 0.73).

243

244 **Two-dimensional micro neutralisation assay:** The two-dimensional micro  
245 neutralisation assay was carried out to find out the antigenic relationship between the  
246 vaccine strains and the field isolates and all the tests were carried out at least twice.  
247 The reactivity of the O<sub>1</sub> Lausanne bvs with the other 23 viruses is shown in Fig 1a.  
248 Eight viruses, including O<sub>1</sub> Manisa had r<sub>1</sub> values of less than 0.3 and four of these (O  
249 ALG 2/99, O ITL 1/93, O TUR 19/91 and O UKG 12/2001) were less than 0.2. The  
250 highest r<sub>1</sub> value for the remaining viruses was 0.88. The r<sub>1</sub> values obtained with the O<sub>1</sub>  
251 Manisa bvs are significantly higher than those for O<sub>1</sub> Lausanne and only two viruses  
252 scored less than 0.3, whilst 14 viruses had r<sub>1</sub> values of 1.0 (Fig 1b).

253

254 **Sequence comparisons:** The virus neutralising O<sub>1</sub> Manisa MAbs employed in this  
255 study are known to be directed against antigenic site 2 with critical residues at  
256 position 72 and 73 in the VP2 capsid protein (Aktas and Samuel, 2000). The viruses  
257 O<sub>1</sub> Lausanne, O ALG 2/99, O BUL 1/93, O GRE 4/94, O SKR 1/2000, O TAI 1/92, O

258 UKG 12/2001, O VIT 4/97 and O VIT 7/97 exhibited poor reactivity with the site 2  
259 Mabs where as the rest of the viruses showed high reactivity. The reason(s) for the  
260 differences in reactivity patterns for various FMD viruses were further investigated by  
261 aligning the sequence of the VP2 region of selected field isolates with that O<sub>1</sub> Manisa  
262 (Fig. 2). The selection of the viruses were based on their reactivity with the site 2  
263 Mabs in O<sub>1</sub> Manisa panel so that viruses exhibiting high reactivity (O PHI 5/95, O  
264 TAW 112/97, O ITL 1/93, O CAM 1/98 and O CAM 6/98) and low reactivity patterns  
265 (O ALG 2/99, O BUL 1/93, O GRE 4/94, O SKR 1/2000, O TAI 1/92, O UKG  
266 12/2001, O VIT 4/97 and O VIT 7/97) were included for this analysis. The known  
267 critical residues of antigenic site 2 at positions 72 and 73 were found to be well  
268 conserved apart from an S-G mutation at position 72 in O TAI 1/92. At position 74,  
269 five isolates had a mutation from P to R/S (see Fig. 2). In addition, certain residues  
270 present adjacent to the critical residues were found to be mutated in certain field  
271 isolates which may have contributed to the reduced reactivity of these MABs. Similar  
272 sequence analyses in relation to Mab binding could not be carried out for the O<sub>1</sub>  
273 Lausanne vaccine strain as the sequence of the vaccine virus is not available.

274

#### 275 **Comparison between the monoclonal and polyclonal antibody based methods:**

276 The antigen homology calculated using the MAb reactivity data was compared with  
277 the r-value data generated from the micro-neutralisation test. In the case of O<sub>1</sub>  
278 Lausanne, the two homology results correlated well with each other except for O<sub>1</sub>  
279 Manisa (virus b in Fig 1a), O TAW 112/97 (virus d in Fig 1a), O ALG 2/99 (virus w  
280 in Fig 1a), O PHI 2/95 (virus i in Fig 1a), O ITL 1/93 (virus j in Fig 1a), O HKN 1/92  
281 (virus k in Fig 1a), O TUR 19/91 (virus c in Fig 1a) and O UKG 12/2001 (virus u in  
282 Fig 1a). For these isolates the antigen homology based on MAb reactivity was found

283 to be high whereas the  $r_1$ -values from the microneutralisation tests had values less  
284 than 0.3, indicating a low match between these isolates and the O<sub>1</sub> Lausanne vaccine  
285 strain.

286

287 In the case of O<sub>1</sub> Manisa, some isolates like O ITL 1/93 (virus j in Fig 1b) and O PHI  
288 9/2000 (virus f in Fig 1b) also showed high antigenic relationship scores based on  
289 MAb reactivity but poor matches (below 0.3) were predicted from the  
290 microneutralisation tests. Conversely, viruses such as O<sub>1</sub> Lausanne (virus a in Fig 1b),  
291 O ALG 2/99 (virus w in Fig 1b), O GRE 4/94 (virus s in Fig 1b), O SKR 1/2000  
292 (virus x in Fig 1b), O TAI 1/92 (virus t in Fig 1b), O UKG 12/2001 (virus u in Fig  
293 1b), O VIT 4/97 (virus q in Fig 1b) and O VIT 7/97 (virus r in Fig 1b) showed the  
294 converse with low antigen homology based on the MAb reactivity but a good match  
295 predicted by microneutralisation ( $r_1$  values ranging between 0.5 and 1.0). Statistical  
296 analysis of the data obtained in this study confirmed the lack of correlation between  
297 the two methods (O<sub>1</sub> Lausanne vaccine: Spearman's rank correlation,  $r=0.14$ ; one-  
298 sided test with alternative hypothesis  $r>0$ ,  $P=0.26$ ); O<sub>1</sub> Manisa vaccine: Spearman's  
299 rank correlation,  $r=-0.002$ ; one-sided test with alternative hypothesis  $r>0$ ,  $P=0.50$ ).

300

### 301 **Discussion:**

302 The reactivity profiles of two panels of MAbs, raised to either O<sub>1</sub> Lausanne or O<sub>1</sub>  
303 Manisa vaccine, against a set of selected type O field isolates were investigated. The  
304 pattern of virus recognition by different MAbs was sometimes similar and sometimes  
305 unique and the observation that MAbs directed towards the same antigenic site (site 2)  
306 could react quite differently indicates the existence of more than one epitope within  
307 the same antigenic site.

308 Antigenic relatedness between the vaccine strains and the field viruses was calculated  
309 using a PAb-based micro-neutralisation test, which is accepted as the gold standard  
310 method for FMD vaccine matching. The MAb-based test was compared with this and  
311 from the data generated the two systems do not correlate well. The PAb data suggest  
312 a broader number of field isolates would be covered well by the O<sub>1</sub> Manisa compared  
313 to O<sub>1</sub> Lausanne strain. This fits with the relative evolutionary relationships of the  
314 vaccines to the field isolates, most isolates being O<sub>1</sub> Manisa like, and of Middle-East /  
315 South Asian origin, whereas O<sub>1</sub> Lausanne is representative of the European / South  
316 American topotype (Samuel and Knowles, 2001). In contrast, the MAb data gives the  
317 opposite result. This may be because of the inclusion of multiple poorly reactive non-  
318 neutralising MAbs whose relevance to the overall response is unclear, or possibly due  
319 to an incomplete panel of O<sub>1</sub> Manisa MAbs which are not fully representative of the  
320 different neutralising antigenic sites. If, for example, one restricts the comparison to  
321 site 2 MAbs (i.e. like-for-like), then the antigenic homology determined by these  
322 MAbs are more similar for the two vaccine strains.

323 The binding sites of the neutralising MAbs used in the O<sub>1</sub> Manisa panel have been  
324 characterised by sequencing MAb neutralisation escape mutants and all of them were  
325 found to be directed towards antigenic site 2 (the BC loop of VP2). This is a  
326 conformational epitope that involves critical amino acid residues at position 72 and  
327 73 (Aktas and Samuel, 2000). Studies using the anti- O<sub>1</sub> Lausanne site 2 MAbs (C6  
328 and C9) have previously identified critical residues on VP2 at positions 70, 71, 75, 77  
329 and 131 in addition to 72 and 73 (Kitson et al, 1990). Comparison of the VP2  
330 sequence of some of the isolates exhibiting low O<sub>1</sub> Manisa Mab reactivity patterns  
331 revealed that these residues are highly conserved and can not therefore account for the  
332 failure of the O<sub>1</sub> Manisa MAbs to strongly react with certain field isolates. Samuel

333 (Samuel, 2000) also reported the sequence at antigenic sites of serotype O FMD virus  
334 to be much conserved, but noted variations at residue VP2<sup>74</sup>. In the current study,  
335 three residues, proline, serine and in one instance arginine were evident at this  
336 position, whilst Samuel (Samuel, 2000) also reported the presence of glutamine.  
337 Apparently mutations at this position do not seem to affect very much the binding of  
338 MAbs. Though amino acid residue at position VP2<sup>131</sup> was found to be highly  
339 conserved, sequence variation was evident in adjacent residues.

340

341 MAb SA107 reacted very well with all isolates whereas the rest of the O<sub>1</sub> Manisa  
342 MAbs showed varying degrees of reactivity. This implies that MAbs SA107 and  
343 SA98 that have been reported to recognise the same epitope (critical amino acid  
344 residue at position 73 (D-A) in the VP2 protein (Aktas and Samuel, 2000) likely have  
345 overlapping but non-identical binding footprints. The reduced reactivity of site 2 O<sub>1</sub>  
346 Manisa MAbs except SA107 with isolate O TAI 1/92 may be attributed to the S-G  
347 mutation at position 72 of VP2 which has been reported to be critical in case of O<sub>1</sub>  
348 Manisa virus (Aktas and Samuel, 2000). Similarly the reduced reactivity of these site  
349 2 O<sub>1</sub> Manisa MAbs with isolates SKR 1/2000 and O UKG 12/2001 were further  
350 investigated. Although these two isolates have a relatively neutral mutation, an  
351 aspartic acid substituted for glutamine at position 133, a more likely explanation for  
352 their decreased reactivity with the MAbs may be the substitution of a positively  
353 charged residue, histidine, at position 79 with the aromatic amino acid residue  
354 tyrosine. None of the isolates that react with SA98 have a change at this residue and  
355 the amino acid residue at position 79 has also been reported to be critical in the case  
356 of FMDV serotype C (Lea et al., 1994) and Asia 1 (Grazioli et al., 2004). This residue  
357 is surface-orientated and presents itself close to the mobile G-H loop of VP1 which

358 may somehow alter the conformation of the epitope resulting in poor reactivity. The O  
359 Vit 3/97 isolate reacted very well with all the O<sub>1</sub> Manisa neutralising MAbs whereas  
360 the other two isolates from Vietnam (O VIT 4/97 and O VIT 7/97) reacted with none  
361 of the neutralising MAbs except SA107. This may be attributed to the presence of a  
362 negatively charged amino acid, glutamic acid (E) at position 134 in place of a  
363 positively charged amino acid, lysine (K). Furthermore, isolates O ALG 2/99 and O  
364 GRE 4/94 also have the same change at this position and both of them exhibited  
365 reduced reactivity with the MAbs indicating amino acid residue at position 134 may  
366 also contribute to the binding of MAbs to serotype O isolates. The residue at position  
367 134 is surface exposed and mutation of this residue to one with a negative charge  
368 might be expected to alter binding efficiency of the MAbs. It is possible that there  
369 may be similar amino acid residue substitution/(s) at other antigenic sites which may  
370 influence the binding of MAbs and have yet not been identified in type O viruses.  
371 This could not be explored further due to the unavailability of multiple MAbs to these  
372 antigenic sites.

373

374 From this study it is clear that though the MAb based system for FMD vaccine  
375 matching is very promising; currently it is neither comparable with the neutralisation  
376 based assay system nor robust enough to be considered as a valuable and  
377 contributable tool for FMD vaccine matching. In order to improve the present system  
378 a wider number of well defined MAbs need to be included in each panel, with at least  
379 one MAb representative of each antigenic site so as to obtain a wider spectrum of  
380 reactivity. In addition considering the evidence of existence of more than one epitope  
381 in some antigenic sites, especially for antigenic site 2, there would therefore be a need  
382 to include more than one MAb for each antigenic site. This may also facilitate

383 identification of amino acid residues influencing the binding of MAbs to other  
384 antigenic sites. Whether the panel of MAbs should consist of only neutralising MAbs  
385 or only non-neutralising MAbs or both, needs to be considered carefully and probably  
386 can only be resolved through validation studies. Considering the ultimate goal of this  
387 system is to measure the reactivity in terms of protection, inclusion of MAbs  
388 recognising protective epitopes seems a sensible approach. However, there is not  
389 enough information available to date to be confident on defining which epitopes these  
390 are. Though a strong correlation has been reported between virus neutralising  
391 antibody and protection in cattle, one of the main target species (Pay and Hingley,  
392 1993) it has also been suggested that non-neutralising epitope/(s) contribute to FMD  
393 vaccine protection (Dunn et al., 1998). However, little or no work has been carried  
394 out so far to identify the epitopes recognised by non-neutralising MAbs and their  
395 contribution to FMD vaccine protection, clearly indicating the urgency to explore  
396 these issues.

397

398 Another possibility to improve the currently used MAb based ELISA system would  
399 be equalisation of the amount of virus captured to the plate. The MAb based system is  
400 an ELISA system which uses a serotype specific polyclonal rabbit antisera as the  
401 capture antibody. Based on the antigenic closeness of the field isolate/(s) with the  
402 vaccine virus (used for raising capture anti-sera) the amount of virus trapped may vary  
403 greatly. Though every effort was made in this study to equalise the amount of virus  
404 trapped by the capture antibody, the possibility of variation could not be totally  
405 excluded. Therefore there is a need to use a universal ligand which may ensure equal  
406 amount of virus being trapped to the system. Work is ongoing in our laboratory to

407 improve the antigen profiling ELISA system using a universal ligand and a wider  
408 panel of MAbs.

409

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415

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417

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502 Figure legend

503

504

505 Fig.1. Antigenic relatedness between the vaccine strains, (a-O1 Lausanne, b- O1

506 Manisa) and type O field isolates. The values for antigen homology calculated from

507 Mab based ELISA are presented as fractions. Closed boxes indicate antigen

508 homology obtained by using MAb based ELISA system and open boxes indicate

509 antigen homology obtained by using PAb based micro neutralisation assay system.

510 For virus names (a-x) on X-axis please see table 2.

511

512 Fig.2. The deduced amino acid sequence of VP2 protein of O1 Manisa vaccine (Aktas

513 et al., 2000) and various serotype O field isolates are aligned as described in materials

514 and methods. Amino acid residues at position 72, 79 and 134 are shown in white with

515 a black background. X –ambiguous.

516

Table 1: The origin and molecular characterisation of foot-and-mouth disease type O viruses used in the study.

Virus	Geographic origin	Species	Date collected	Topotype	Strain*
O <sub>1</sub> /Lausanne/SWI/65	Lausanne, Switzerland.	not known	1965	EURO-SA	-
O <sub>1</sub> /Manisa/TUR/69	Manisa, Turkey.	bovine	Apr, 1969	ME-SA	-
O/ALG/2/99	Bir touta, Blida, Algeria.	bovine	1999	WA	-
O/BUL/1/93	Simeonovgrad, Bulgaria.	bovine	1993	ME-SA	-
O/CAM/3/92	Banon, Battambang, Cambodia.	bovine	31/03/1992	SEA	-
O/CAM/1/98	Kâmpóng Speu, Cambodia.	porcine	05/01/1998	SEA	Cam-94
O/CAM/6/98	Kâmpóng Speu, Cambodia.	porcine	08/01/1998	SEA	Cam-94
O/CAM/8/98	Kâmpóng Speu, Cambodia.	porcine	01/08/1998	SEA	Cam-94
O/GRE/4/94	Xanthi, Greece.	bovine	01/07/1994	ME-SA	-
O/HKN/1/92	Maon Shan, Shatin, Hong Kong.	porcine	22/01/1992	CATHAY	-
O/ITL/1/93	Italy.	bovine	22/02/1993	ME-SA	-
O/PHI/2/95	Baunag(Bauang?), La Union, Region I, Philippines.	porcine	12/01/1995	CATHAY	-
O/PHI/9/95	Kapasigan, Pasig City, NCR, Philippines.	porcine	23/03/1995	CATHAY	-
O/PHI/1/99	Mecauyan, Bulacan, Region III, Philippines.	Porcine	1999	CATHAY	-
O/PHI/9/2000	Pritil, Guiguinto, Bulacan, Region III, Philippines.	porcine	03/01/2000	CATHAY	-
O/PHI/23/2000	San Andres, Cainta, Rizal, Region IV, Philippines.	porcine	24/05/2000	CATHAY	-
O/SKR/1/2000	Papyung, P'aju City, Kyunggi, Republic of Korea.	bovine	26/03/2000	ME-SA	PanAsia
O/TAW/112/97	Sinchu Prefecture, Taiwan.	porcine	07/12/1997	CATHAY	-
O/TAI/1/92	Narathiwat, Thailand.	not known	1992	SEA	-
O/TUR/19/91	Savas, Aksehir, Konya, Turkey.	bovine	12/09/1991	ME-SA	-
O/UKG/12/2001	Brentwood, Essex, UK.	porcine	20/02/2001	ME-SA	PanAsia
O/VIT/3/97	Vietnam.	porcine	1997	CATHAY	-
O/VIT/4/97	Vietnam.	bovine	1997	SEA	Cam-94
O/VIT/7/97	Uar, Krong Pa, Gialai, Region no. 37, Vietnam.	not known	31/08/1997	SEA	Cam-94

\*, strain name given where designated.

Topotype designations are as follows: EURO-SA, Europe-South America; ME-SA, Middle East-South Asia; SEA, Southeast Asia; WA, West Africa

Virus names are given as WRLFMD codes and the codes are as follows: ALG, Algeria; BUL, Bulgaria; CAM, Cambodia; GRE, Greece; HKN, Hong Kong; ITL, Italy; PHI, Philippines; SKR, South Korea (Republic of Korea); SWI, Switzerland; TAI, Thailand; TAW, Taiwan Province of China; TUR, Turkey; UKG, United Kingdom; VIT, Vietnam.

Table 2: Critical residues of type O MAbs

Mab	Site	Critical residue
<u>O<sub>1</sub> Lausanne MAbs</u>		
B2	1	VP1- 144, 148, 154
D9	1	VP1 -144
C6	2	VP2 – 70, 71, 73
C9	2	VP2 -71, 72, 75, 77
C8	3	VP1- 43, 44
OC3	5	VP1- 149
<u>O<sub>1</sub> Manisa MAbs</u>		
SA85	2&5	VP2-72, VP1-149
SA88	2	ND
SA98	2	VP2-73
SA107	2	VP2-73
SA113	2	VP2-72
SA120	2	ND
SA127	2	VP2-73

ND- Not determined

Table 3 Reactivity of foot-and-mouth disease virus serotype O field isolates with O1 Lausanne MAbs.

	Virus Name	B2	D9	C6	C9	C8	OC3
a	O1 Lausanne						
b	O1 Manisa						
c	O TUR 19/91						
d	O TAW 112/97						
e	O PHI 23/2000						
f	O PHI 9/2000						
g	O PHI 1/99						
h	O PHI 9/95						
i	O PHI 2/95						
j	O ITL 1/93						
k	O HKN 1/92						
l	O CAM 8/98						
m	O CAM 6/98						
n	O CAM 1/98						
o	O CAM 3/92						
p	O VIT 3/97						
q	O VIT 4/97						
r	O VIT 7/97						
s	O GRE 4/94						
t	O TAI 1/92						
u	O UKG 12/2001						
v	O BUL 1/93						
w	O ALG 2/99						
x	O SKR 1/2000						

Reactivity Scale

	0-20%
	21-50%
	51-100%

Table 4: Reactivity of foot-and-mouth disease virus serotype O field isolates with anti-O1 Manisa MAbs. For reactivity scale see Table 1.

	Virus name	SA107	SA120	SA113	SA127	SA85	SA88	SA98	3H3	4B12	3A4	4C8	4H6	3G3
a	O1 Lausanne	■	■	■	■	■	■	■	■	■	■	■	■	■
b	O1 Manisa	■	■	■	■	■	■	■	■	■	■	■	■	■
c	O TUR 19/91	■	■	■	■	■	■	■	■	■	■	■	■	■
d	O TAW 112/97	■	■	■	■	■	■	■	■	■	■	■	■	■
e	O PHI 23/2000	■	■	■	■	■	■	■	■	■	■	■	■	■
f	O PHI 9/2000	■	■	■	■	■	■	■	■	■	■	■	■	■
g	O PHI 1/99	■	■	■	■	■	■	■	■	■	■	■	■	■
h	O PHI 9/95	■	■	■	■	■	■	■	■	■	■	■	■	■
i	O PHI 2/95	■	■	■	■	■	■	■	■	■	■	■	■	■
j	O ITL 1/93	■	■	■	■	■	■	■	■	■	■	■	■	■
k	O HKN 1/92	■	■	■	■	■	■	■	■	■	■	■	■	■
l	O CAM 8/98	■	■	■	■	■	■	■	■	■	■	■	■	■
m	O CAM 6/98	■	■	■	■	■	■	■	■	■	■	■	■	■
n	O CAM 1/98	■	■	■	■	■	■	■	■	■	■	■	■	■
o	O CAM 3/92	■	■	■	■	■	■	■	■	■	■	■	■	■
p	O VIT 3/97	■	■	■	■	■	■	■	■	■	■	■	■	■
q	O VIT 4/97	■	■	■	■	■	■	■	■	■	■	■	■	■
r	O VIT 7/97	■	■	■	■	■	■	■	■	■	■	■	■	■
s	O GRE 4/94	■	■	■	■	■	■	■	■	■	■	■	■	■
t	O TAI 1/92	■	■	■	■	■	■	■	■	■	■	■	■	■
u	O UKG 12/2001	■	■	■	■	■	■	■	■	■	■	■	■	■
v	O BUL 1/93	■	■	■	■	■	■	■	■	■	■	■	■	■
w	O ALG 2/99	■	■	■	■	■	■	■	■	■	■	■	■	■
x	O SKR 1/2000	■	■	■	■	■	■	■	■	■	■	■	■	■

Fig. 1 Comparison of antigen homology

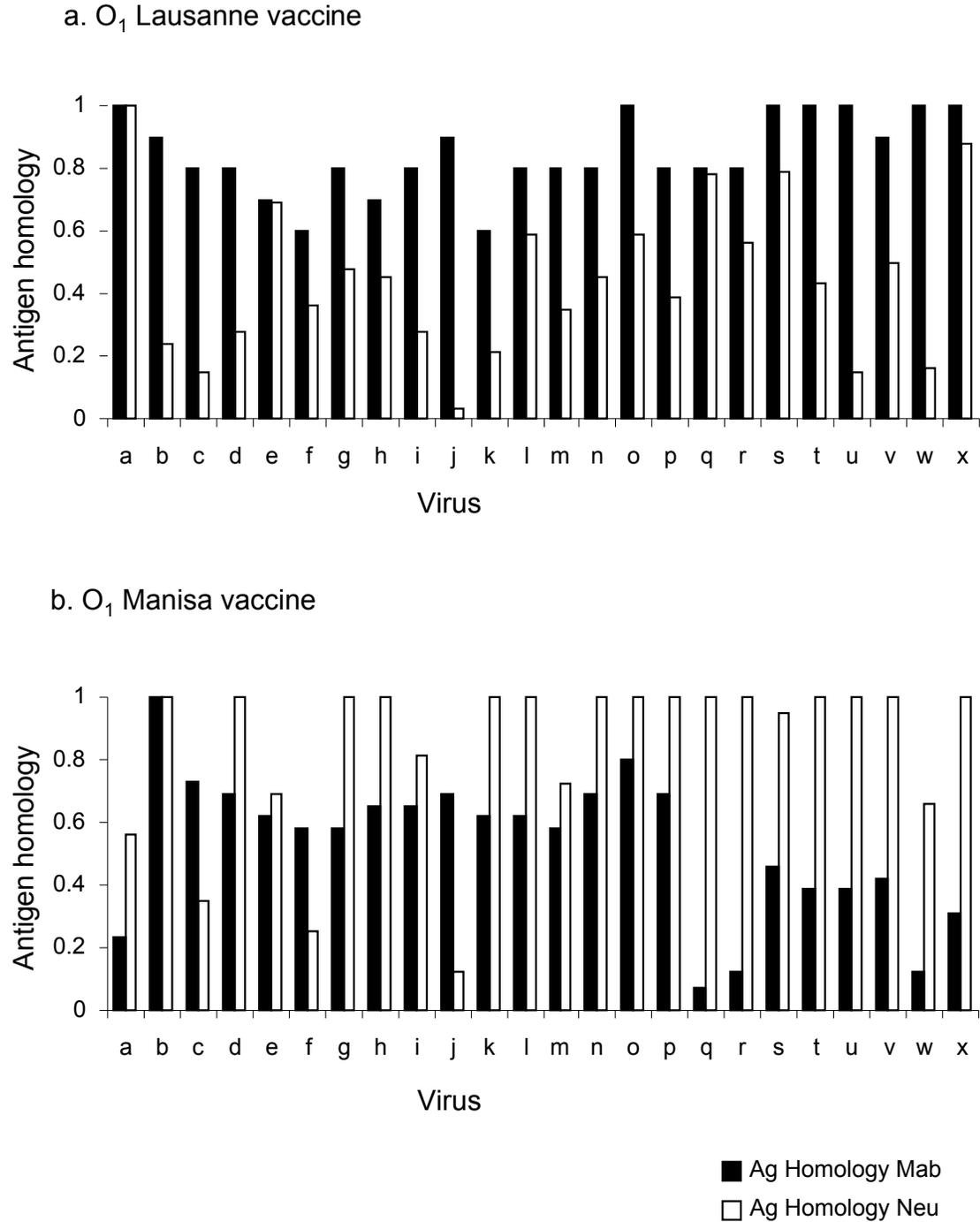


Fig. 2 Sequence comparison of VP2 region of various FMD viruses

	10	20	30	40	50	60
MANISA	DKKTEETTLLEDRI	LTRNGHTTSTTQ	SSVGVTYGYATA	EDFVSGPNTSG	LETRVAQAER	
TAW97-112	-----	-----	-----	-----	-----	V----
PHI95-05	-----	-----	-----	-----	-----	V----
ITL93-01	-----	-----	-----	-----	-----	V----
CAM98-01	-----	-----	-----	-----	-----	V----
CAM98-06	-----	-----	-----	-----	-----	V----
TAI92-01	-----	-----	-----	I-----	-----	V----
VIT97-03	-----	-----	-----	-----	-----	V----
VIT97-04	-----	-----	-----	-----	-----	V----
VIT97-07	-----	-----	X-----	X-----	X-----	V-E--
ALG99-02	-I-----	-----	-----	-----	-----	V----
GRE94-04	-----	A-----	-----	-----	S-----	V----
SKR00-01	-----	-----	-----	-----	-----	V----
UKG01-12	-----	-----	-----	-----	-----	V----

	70	80	90	100	110	120
MANISA	FFKTHLFDWVTS	DPFGRCHLLE	LPTDHKGVYGS	LTDSYAYMRNG	WDVEVTAVGN	QFNNGGC
TAW97-112	-----	-----	-----	-----	G-----	I-----
PHI95-05	-----	-----	-----	-----	-----	-----
ITL93-01	-----	-----	-----	-----	-----	-----
CAM98-01	-----	S-----	-----	N-----	-----	-----
CAM98-06	-----	S-----	-----	N-----	-----	-----
TAI92-01	-----	AG-----	-----	-----	-----	-----
VIT97-03	-----	-----	-----	-----	-----	-----
VIT97-04	-----	A--S-----	-----	-----	-----	-----
VIT97-07	-----	S-----	-----	-----	-----	-----
ALG99-02	-----	-----	-----	-----	-----	-----
GRE94-04	-----	R-----	-----	G-----	-----	-----
SKR00-01	-----	-----	-----	-----	-----	-----
UKG01-12	-----	-----	-----	-----	-----	-----

	130	140	150	160	170	180
MANISA	LLVAMVPELCSI	QKRELYQLTL	FPHQFINPRTN	MTAHITVPFV	GVNRYDQYK	VHKPWTLV
TAW97-112	-----	S-----	-----	-----	YL-----	-----
PHI95-05	-----	R--N-----	-----	-----	A-YL-----	-----
ITL93-01	-----	-----	-----	-----	-----	-----
CAM98-01	-----	LER-----	-----	-----	S-----	-----
CAM98-06	-----	LER-----	-----	-----	S-----	-----
TAI92-01	-----	-----	-----	-----	K-----	-----
VIT97-03	-----	S-----	-----	-----	YL-----	-----
VIT97-04	-----	LKE--K-----	-----	-----	S-----	-----
VIT97-07	-----	LKE--K-----	-----	-----	S-----	-----
ALG99-02	-----	E-----	-----	-----	-----	Q-----
GRE94-04	-----	V-E-----	-----	-----	-----	I-----
SKR00-01	-----	D-----	-----	-----	-----	-----
UKG01-12	-----	D-----	-----	-----	-----	-----

	190	200	210
MANISA	VMVVAPLTVN	SEGAPQIKVY	ANIAPTNVHV
TAW97-112	-----	N-----	L-----
PHI95-05	-----	N-----	L-----
ITL93-01	-----	T-----	-----
CAM98-01	-----	T-----	L-----
CAM98-06	-----	T-----	L-----
TAI92-01	-----	T-----	-----
VIT97-03	-----	N-----	L-----
VIT97-04	-----	T--Q-----	L-----
VIT97-07	-----	T--Q-----	L-----
ALG99-02	-----	N-----	L-----
GRE94-04	-----	N-----	-----
SKR00-01	-----	T-----	-----
UKG01-12	-----	T-----	-----