Serological response of guinea pigs to inactivated 146S antigens of foot and mouth disease virus after single or repeated inoculations

N.P. FERRIS and A.I. DONALDSON*

Summary: Guinea pig antiserum production following administration of inactivated foot and mouth disease virus 146S antigens was found to be acceptable for routine use in the complement fixation typing test, within defined criteria. The specificity of antisera after the single or repeated inoculation regimes was strain dependent, probably reflecting a variation in antigenic stability. Antisera with a narrow intra-typic response were shown to be usable in the typing test by making mixtures.

INTRODUCTION

The World Reference Laboratory for foot and mouth disease virus (FMDV) routinely uses the complement fixation (CF) test for the identification (typing) of field isolates. Further serological investigation is done by the virus neutralisation test. In the past the antisera have generally been prepared in guinea pigs by hyperimmunisation with live virus (1). Recently we have tested procedures for producing inactivated 146S antigens of FMDV (7) and in this paper we have evaluated the serological response of guinea pigs inoculated with these antigens by single and repeated inoculations.

MATERIALS AND METHODS

Inactivated antigen preparation.

Virus growth, inactivation and purification have been described previously (7).

Antiserum production.

Anti-146S sera were produced by either single or repeated inoculations of inactivated 146S antigens into guinea pigs.

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For the single dose procedure, the guinea pigs were inoculated subcutaneously with 10 µg of inactivated 146S particles mixed with an equal volume of Freund's complete adjuvant (FCA) and killed and exsanguinated after 28 days.

For the repeated inoculation regime, 5 µg doses of inactivated 146S particles were administered at an interval of 30 days (the first mixed with an equal volume of FCA and the second with incomplete adjuvant). The animals were killed and exsanguinated 15 days after the second inoculation. In the majority of cases, antigens had been stored above liquid nitrogen for long periods (months) prior to commencement of this regime.

The sera were heat-inactivated at 56°C for 30 min, pooled, Seitz filtered and stored at −20°C.

Hyperimmune guinea pig antisera, prepared by the method of Brooksby (1), were obtained from sources within the Institute. In this case, guinea pigs had been inoculated with live guinea pig-adapted-virus, left for 2 to 3 months, and were then hyperimmunised with fresh antigen from guinea pig footpad suspension. Ten days later they were killed and exsanguinated.

Cross-specificity analysis.

The type specificity of each antiserum was investigated using a micro-complement fixation test (CF; 3). Antiserum was diluted in veronal buffer (VBD) in 1.5 fold dilution steps from an initial 1 in 16 dilution to leave 25 µl of successive antiserum dilutions in wells across the microtitre plate. 50 µl of 3 units of complement were then added, followed by 25 µl of virus suspension. Virus suspensions were grown in monolayer cultures of BHK-21 cells, harvested when cultures showed maximal CPE and clarified by centrifugation. Antigens were either used unconcentrated or concentrated by precipitation with polyethylene glycol and resuspended in a 50-fold reduced volume of 0.05 M tris buffer containing 0.1 M sodium chloride, pH 7.6. The optimal dilution of each antigen preparation was determined in a chequerboard format against the homologous antiserum. Both unconcentrated and the optimum homotypic dilution of concentrated antigens were used to assess heterotypic CF reactions.

Incubation of the test system took place at 37°C for 1 hour prior to the addition of 25 µl of 1.4% standardised sheep red blood cells (SRBC) in VBD sensitisised with 5 units of rabbit anti-SRBC. The reagents were incubated at 37°C for a further 30 min and the plates subsequently centrifuged. CF titres were expressed as the reciprocal of the serum dilution producing 75% haemolysis.

Specificity of homotypic antiserum mixtures.

Mixtures of three separate strains of both type A and type Asia 1 guinea pig anti-146S sera were made by combining 50 µl of each antiserum with
2,250 µl of VBD. CF tests were then performed with the original range of heterotypic antigens and a wider selection of homotypic strains against both the mixtures and the individual constituent antisera. CF titres of the mixtures were calculated on a 1 in 16 starting dilution basis.

RESULTS

Antiserum specificity.

The highest CF titres of the guinea pig anti-146S sera obtained with both unconcentrated antigens and the optimum dilutions of concentrated antigens are shown in Tables I and II. An acceptable serum was defined as one which had an homologous titre of ≥ 122 and no heterotypic reactivity closer in titre than 5 dilution steps below. By these criteria, the majority of antigens stimulated an acceptable response when delivered in a single inoculation. Others were of lower type specificity and a few unacceptable, e.g. O IND 53/79 (a), SAT 2 R1215 and SAT 3 BEC 1/65. Antiserum to A₅ Allier, A₂₂ Mahmatli and A₃₀ Pando were almost totally strain specific being of high titre to antigen of the homologous strain but showing a CF reaction against other homotypic strains of the same order as heterotypic fixation.

Homotypic antiserum titres were generally increased when guinea pigs were inoculated with antigens on two successive occasions. However, this regime also tended to increase the heterotypic cross-reaction (with the exception of the antiserum to O₁ BFS 1860 which was totally type-specific). Antiserum to O₁ Lausanne, O IND 53/79 and SAR 3/79 showed particularly high heterotypic fixation which made them unacceptable as typing reagents.

Three out of the nine hyperimmune guinea pig sera tested produced strong heterotypic fixation (Table III). The two type C antisera reacted strongly with O and A antigens; C Noville also reacted with Asia 1 antigens. Antiserum to Israel 3/63 fixed complement with antigen of every type.

Antiserum mixtures.

An essential requirement of a CF typing reference antiserum is that it should have a broad spectrum intra-typic reactivity. It is well recognised and is also evident from Tables I, II and III that certain strains fulfil this requirement better than others e.g. from Table I, A₅ Allier, A₂₂ Mahmatli and A₃₀ Pando were strain rather than type specific. Mixtures of homotypic antisera were made and retested for cross-reactivity to investigate if this would overcome the problem of narrow intra-typic reactivity.

The CF reactions of type A and Asia 1 antiserum mixtures and their homotypic constituent parts tested individually against an increased range of homotypic strains are shown in Tables IV and V. The antiserum to A₂₂ Mahmatli, in the type A pool, was almost totally strain specific; A₅ Allier antiserum reacted against a greater number of subtypes but to a lesser extent than
| Antiserum | \(O_1\) Lausanne | \(O_1\) BFS 1860 | \(O_2\) Brescia | \(O_5\) IND 1/62 | \(O_5\) IND 1979 (a) | \(O_5\) IND 1979 (b) | A5 Allier | A22 Mahmatli | A24 Cruzeiro | A29 Pando | C1 Noville | C2 Resende | SAT 1 T 155/71 | SAT 2 KEN 183/74 | SAT 2 SAR 3/79 | SAT 3 BEC 1/65 | Asia 1 CAM 9/80 | Asia 1 Turkey 1973 |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------|------------|-------------|-----------|------------|------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| \(O_1\) Lausanne | 618 | 122 | 122 | 54 | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| \(O_1\) BFS 1860 | 183 | 275 | 81 | 81 | 36 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| \(O_2\) Brescia | 81 | 54 | 183 | 36 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| \(O_5\) IND 1/62 | 275 | 183 | 275 | 275 | 275 | 24 | 16 | 16 | 16 | 0 | 24 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| \(O_5\) IND 1979 (a) | 54 | 183 | 183 | 122 | 183 | 54 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 0 |
| \(O_5\) IND 1979 (b) | 275 | 275 | 183 | 122 | 275 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 0 |
| A5 Allier | 0 | 0 | 0 | 0 | 0 | 183 | 0 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A22 Mahmatli | 0 | 0 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A29 Pando | 0 | 0 | 16 | 0 | 16 | 36 | 36 | 927 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C1 Noville | 0 | 0 | 16 | 0 | 16 | 36 | 36 | 36 | 0 | 0 | 122 | 81 | 0 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C2 Resende | 24 | 24 | 24 | 36 | 16 | 24 | 16 | 122 | 412 | 24 | 24 | 24 | 16 | 16 | 16 | 36 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 0 |
| SAT 1 T 155/71 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SAT 2 KEN 183/74 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SAT 2 R 1215 | 24 | 24 | 36 | 36 | 24 | 36 | 36 | 54 | 24 | 54 | 36 | 36 | 81 | 36 | 36 | 16 | 36 | 36 | 36 | 24 | 0 | 0 | 0 |
| SAT 2 SAR 3/79 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| SAT 3 BEC 1/65 | 24 | 24 | 36 | 36 | 24 | 24 | 16 | 16 | 16 | 16 | 0 | 24 | 24 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 0 |
| Asia 1 CAM 9/80 | 0 | 16 | 24 | 16 | 0 | 0 | 24 | 16 | 0 | 24 | 16 | 24 | 16 | 0 | 0 | 16 | 412 | 122 | 122 | 81 | 0 | 0 | 0 | 0 |
| Asia 1 IND 8/79 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 81 | 24 | 36 | 412 | 0 | 0 | 0 |
| Asia 1 PAK 1/54 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 54 | 183 | 927 | 16 | 0 | 0 | 0 |
| Asia 1 Turkey 1973 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 16 | 36 | 16 | 24 | 16 | 24 | 16 | 24 | 36 | 24 | 24 | 54 | 412 | 412 | 24 | 0 | 0 |

* Expressed as the reciprocal of the serum dilution producing 75% haemolysis.
TABLE II

**CF titres* in antiserum of guinea pigs inoculated twice with inactivated 146S antigens**

<table>
<thead>
<tr>
<th>Virus</th>
<th>O1 Lausanne</th>
<th>O1 BFS 1860</th>
<th>O2 Brescia</th>
<th>O4 IND 1/62</th>
<th>O IND 53/79</th>
<th>A5 Allier</th>
<th>A22 Mahmatli</th>
<th>A24 Cruzeiro</th>
<th>C1 Noville</th>
<th>C3 Resende</th>
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<th>SAT 2 KEN 183/74</th>
<th>SAT 2 SAR 3/79</th>
<th>SAT 3 BEC 1/65</th>
<th>Asia 1 CAM 9/80</th>
<th>Asia 1 Turkey 1973</th>
<th>Asia 1 PAK 1/54</th>
<th>Asia 1 IND 8/79</th>
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* Expressed as the reciprocal of the serum dilution producing 75% haemolysis.
| Table III |
|CF titres* of hyperimmune guinea pig sera |

**Antiserum**

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<th>Virus</th>
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<th>O1 BFS 1860</th>
<th>O2 Brescia</th>
<th>O5 IND 1/62</th>
<th>O IND 53/79</th>
<th>A5 Allier</th>
<th>A22 Mahmatli</th>
<th>A24 Cruzeiro</th>
<th>A30 Pando</th>
<th>C1 Noville</th>
<th>C3 Resende</th>
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<th>SAT 2 SAR 3/79</th>
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* Expressed as the reciprocal of the serum dilution producing 75% haemolysis.
### TABLE IV
Specificity of a mixture of three type A guinea pig anti-146S strain sera in CF tests

**(a) Homotypic reaction**

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>A₂ Spain/43</th>
<th>A₃ GFR/44</th>
<th>A₄ GFR/48</th>
<th>A₅ Allier</th>
<th>A₆ Italy/50</th>
<th>A₇ Spain/59</th>
<th>A₈ THAIL/60</th>
<th>A₉ USSR/60</th>
<th>A₁₀ KEN/64</th>
<th>A₁₁ Mahmatli</th>
<th>A₁₂ CRUZ</th>
<th>A₁₃ PANDO</th>
<th>A₁₄ VEN/70</th>
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<td>(1) Mixture*</td>
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<td>618</td>
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<td>122</td>
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<td>(2) A₅ Allier</td>
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<td>122</td>
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<td>412</td>
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<td>122</td>
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**(b) Heterotypic reaction**

<table>
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<tr>
<th>Antiserum</th>
<th>O₁ Luosanne</th>
<th>O₁ BFS 1860</th>
<th>O₂ IND 53/79</th>
<th>C₁ Noville</th>
<th>C₂ Resende</th>
<th>SAT 1 T 155/71</th>
<th>SAT 2 KEN 183/74</th>
<th>SAT 2 SARS 3/79</th>
<th>SAT 3 BEC 1/65</th>
<th>Asia 1 CAM 9/80</th>
<th>Asia 1 Turkey 1973</th>
<th>Asia 1 PAK 1/54</th>
<th>Asia 1 IND 8/79</th>
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</tbody>
</table>

* Equal amounts of (2), (3) and (4); total serum volume diluted 1 in 16 with VBD.

** CF titre; titre calculated on initial 1 in 16 dilution basis.
**TABLE V**

*Specificity of a mixture of three type Asia I guinea pig anti-146S strain sera in CF tests*

(a) **Homotypic reaction**

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>1973</th>
<th>CAM 9/80</th>
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<th>PAK 1/54</th>
<th>275</th>
<th>IND 8/79</th>
<th>81</th>
<th>KEN 3/63</th>
<th>122</th>
<th>BUR 8/82</th>
<th>81</th>
<th>KEN 2/81</th>
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<th>OMA 2/82</th>
<th>81</th>
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<td>(4) IND 8/79</td>
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</table>

(b) **Heterotypic reaction**

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>O1, Lausanne</th>
<th>O1, BFS, 1860</th>
<th>O-IND, 53/79</th>
<th>A2 Allier</th>
<th>A22 Mahmati</th>
<th>A24, Cruzeiro</th>
<th>C1, Noville</th>
<th>C3, Resende</th>
<th>SAT 1T 153/71</th>
<th>SAT 2 KEN 183/74</th>
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</tbody>
</table>

* Equal amounts of (2), (3) and (4); total serum volume diluted 1 in 16 with VBD.

** CF titre; titre calculated on initial 1 in 16 dilution basis.
did A$_{24}$ Cruzeiro which had the broadest reaction spectrum. The pool reacted to an acceptable degree with all subtypes with the exception of A$_{30}$ Pando and without an increase in amount of heterotypic reaction. None of the antisera reacted strongly with A$_{30}$ Pando but inclusion of antiserum to this virus in the antiserum mixture increased its strain reaction in the same way as did A$_{22}$ Mahmatli (unpublished results). Antiserum to IND 8/79 (Table V) had a narrower range of reaction with other Asia 1 strains then either CAM 9/80 or PAK 1/54. The Asia 1 mixture produced a comprehensive coverage of strains and had very low cross-typic reactivity.

**DISCUSSION**

The essential requirements of a serum for typing FMDV isolates by CF are that it (i) has a high homologous CF titre; (ii) has a broad homotypic reaction, and (iii) exhibits no or a very low heterotypic reaction. In the past the recommended procedure for the production of sera for use in such tests has been carried out by the hyperimmunisation of guinea pigs with live virus adapted to that species (1). This procedure generally produces high titred sera but has disadvantages:

(a) Requirement (iii) is not always fulfilled (2). A crude harvest of FMDV from guinea pig material will contain 12S and VIA antigens as well as 146S antigen; serum produced by the hyperimmunisation of such material will contain antibodies reactive with 12S and VIA antigens in addition to antibodies reactive with the 146S component. The presence of high percentage amounts of the former antibodies is undesirable as VIA antibody is not type specific and 12S antibody is cross-reactive (10).

(b) It has been shown that continued passage of FMDV in vitro can cause changes in antigenic properties leading to the production of a less efficient vaccine (6, 8). Similarly the adaptation of FMDV strains to growth in guinea pigs could lead to the selection of minor variants from field virus populations and thence to a non-representative antiserum.

The use of inactivated antigens for the preparation of reference antisera offers certain advantages: (i) it provides more reproducible methods for their production; (ii) inactivation yields antigens which are safe for handling within a laboratory and for transportation between laboratories; (iii) groups of guinea pigs inoculated with different types of FMDV antigens can be housed in the same room; and (iv) in the absence of either convalescent or post-vaccinal antisera from livestock, the use of sera which are more compatible with those induced by vaccines is preferable for the provision of advice on the immunological suitability of available vaccines for emergency control of outbreaks (10).

The disadvantages of this method are: (a) inactivation usually reduces the amount of 146S particles obtained after purification. The degree of reduction may vary from slight, e.g. Asia 1 CAM 9/80, to marked, e.g.
SAT 1 T 155/71 (7 and Ferris, unpublished results); (b) the safe use of ethyleneimine derivatives requires special handling facilities.

In the present study it was found that antisera raised by the single inoculation regime generally had an acceptable level of CF antibody although often lower than hyperimmune sera. The level of heterotypic CF reaction was generally low, with the exceptions described; probably reflecting variation in the immunogenic stability of the various antigens used for guinea pig inoculation. For example, it proved difficult to produce inactivated 146S antigen to SAT 2 R1215 (7) and that obtained may have been degraded to such an extent as to broaden its reactivity leading to antisera exhibiting spurious heterotypic fixation.

Double inoculations stimulated high CF titres to levels compatible with hyperimmune sera. This method usually increased the degree of cross-typic reaction which in three instances was to an unacceptable level. It is possible that degradation of 146S antigens took place following inoculation leading to the production of antibody to the 12S component. Cowan and Graves (5) reported that guinea pig serum raised against inactivated FMDV antigen does not contain VIA-antibody; however, Pinto and Garland (9) found that multiple vaccination of cattle with formalin-inactivated FMDV vaccine can result in VIA-antibody formation. It has not been tested as to whether any cross-reactions with our guinea pig sera are due to VIA-antibody. The rejection rate for antisera produced by this method was no higher than by the hyperimmunisation method. In other cases the dilution of sera with very high homologous titres could reduce the heterologous cross-reaction to the minimum acceptable level defined.

Specific antisera have been produced by absorption with heterologous FMDV (2, 4). This method is hampered by the fact that relatively large amounts of virus antigen are required; often the homologous CF titre is markedly reduced and the results are not always reproducible. An alternative approach may be to inhibit heterotypic antibody formation by the passive administration of antibody (11). This procedure has been shown to improve the type specificity of FMDV antiserum although it may again reduce the homologous CF titre (Osborne, personal communication). The results illustrate that certain FMDV antigens induce sera of very narrow specificity whereas others tend to produce a broader spectrum of reaction; this emphasises the necessity of adequate cross-specificity testing before a serum is put into routine use. In instances where a serum is of a too narrow intra-typic reactivity it may be possible to correct this by using mixtures.

This was illustrated in the current investigations in the case of the type A and Asia 1 antiserum mixtures made. By careful selection, manipulation of mixture volumes and testing of antisera, a pooled preparation could be obtained which should cover any expected subtype within a type.

These results demonstrate that the production of antisera from inactivated antigens is an acceptable procedure. Whether single or repeated inocula-
tion procedures are used may depend upon the immunogenic stability characteristics of the individual FMDV strain. With particularly unstable strains, live virus may have to be utilised for antiserum production. The resulting serum may have use either for typing or subtyping or both depending upon its titre and extent of cross-reactivity.

ACKNOWLEDGEMENTS

We should like to thank Mrs R.M. Philpot, Mrs M. MacDougall, Miss S.M. Heugh, Miss N.E. Gostling, L. Pullen and E. Chapman for their excellent technical assistance.

*  
* *

RÉACTION SÉROLOGIQUE DES COBAYES A L’INOCULATION SIMPLE OU RÉPÉTÉE D’ANTIGÈNES 146S INACTIVÉS DU VIRUS APHTEUX. — N.P. Ferris et A.I. Donaldson.

Résumé : Les antisérums produits par inoculation aux cobayes d’antigènes 146S inactivés du virus aphteux ont été reconnus acceptables pour le typage de routine du virus par l’épreuve de fixation du complément, dans les limites de critères bien définis. Après inoculation simple ou répétée, les antisérums se sont montrés plus ou moins spécifiques selon les souches du virus, ce qui traduit probablement une variation de la stabilité antigénique. Les antisérums ayant un spectre étroit de réactivité à l’intérieur d’un même type peuvent être utilisés pour l’épreuve de typage en les mélangant à d’autres.

*  
* *

REACCIÓN SEROLÓGICA DE LOS COBAYOS EN LA INOCULACIÓN SIMPLE O REPETIDA DE ANTÍGENOS 146S INACTIVADOS DEL VIRUS AFTOSO. — N.P. Ferris y A.I. Donaldson.

Resumen : Los antisueros producidos por inoculación en los cobayos de antígenos 146S inactivados del virus aftoso fueron reconocidos aceptables para la tipificación rutinaria del virus con la prueba de fijación del complemento, dentro de los límites de criterios bien definidos. Tras la inoculación simple o repetida, los antisueros se mostraron más o menos específicos en función de las cepas del virus, con lo que probablemente se pone de manifiesto una variación de la estabilidad antigénica. Se pueden utilizar antisueros con un espectro estrecho de reactividad dentro de un mismo tipo para la prueba de tipificación mezclándolos con otros.

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* *
REFERENCES


