

## **The use of the haemagglutination-inhibition test for detecting antibodies to type SAT 2 foot-and-mouth disease viruses in cattle sera**

BY J. C. BOOTH AND T. W. F. PAY

*The Wellcome Foot and Mouth Disease Vaccine Laboratory, Pirbright, Surrey*

AND R. S. HEDGER AND I. T. BARNETT

*The Animal Virus Research Institute, Pirbright, Surrey*

(Received 12 August 1974)

### SUMMARY

Two strains of type SAT 2 foot-and-mouth disease virus which gave high titres of haemagglutinin activity reacted type-specifically in direct haemagglutination-inhibition tests with reference, bovine convalescent antisera. Comparisons of the haemagglutination-inhibition and the serum neutralization tests using cattle sera showed that both were equally specific and sensitive for detecting virus antibody.

### INTRODUCTION

Several serological techniques for measuring antibody titres to foot-and-mouth disease (FMD) viruses in the sera of cattle and other ungulates have been developed and evaluated. These include, among others, the passive haemagglutination test (Reda & Wittmann, 1972; Tokuda & Warrington, 1970; Warrington & Kawakami, 1972); the indirect complement-fixation test (De Simone, Lodetti, Panina & Nardelli, 1970); the single radial diffusion test (Cowan & Wagner, 1970; Wagner, Cowan & McVicar, 1972) and, most widely used and accepted of all, the serum neutralization test (Hedger, Condy & Golding, 1972; Lodetti, De Simone & Nardelli, 1972; Martin & Chapman, 1961; Wagner & McVicar, 1970). However, the recent demonstration that type SAT 2 FMD viruses are able to agglutinate guinea-pig erythrocytes directly (Booth & Pay, 1973), has made it possible to consider the direct haemagglutination-inhibition (HI) test as yet another method for assaying antibody activity to these viruses. Accordingly, the present report deals with comparisons of the sensitivity and the specificity of the HI and the serum neutralization tests for detecting antibodies to type SAT 2 FMD viruses in cattle sera.

### MATERIALS AND METHODS

#### *Cattle sera*

Reference convalescent bovine antisera to all seven immunological types of FMD virus were obtained from the Serum Assay Section, Animal Virus Research Institute, Pirbright.

Sera from vaccinated cattle were obtained in the course of a field trial on a batch of experimental, inactivated SAT 2 Uga 6/70 virus vaccine prepared at the Vaccine Research Department, Animal Virus Research Institute, Pirbright; also during potency tests on monovalent, inactivated SAT 2 Tan 5/68 and SAT 2/3 Ken 3/57 virus vaccines prepared by the Wellcome Foundation Ltd. Samples of blood were taken from the animals immediately before vaccination and at intervals thereafter up to 49 days. In all cases a second dose of vaccine was administered at 21 days after the primary injection.

#### *Haemagglutination (HA) test*

Virus antigens were grown in rolled cultures of BHK 21 cells maintained under Eagle's medium containing twice the standard concentrations of amino acids and vitamins, 10% tryptose phosphate broth and antibiotics. The infected culture fluids were harvested when the cell sheets had stripped from the glass and were freed of gross cellular debris by centrifuging at 2000 rev./min. for 15 min. in the M.S.E. 4L centrifuge, then at 10,000 rev./min. for 30 min. in the M.S.E. 8 × 50 ml. angle rotor. The resulting clarified suspension of virus was then centrifuged at 30,000 rev./min. for 60 min. in the same rotor to concentrate the virus particle haemagglutinins (by pelleting them) and, at the same time, rid them of the non-haemagglutinating, slowly sedimenting, sub-viral components usually present in tissue culture harvests of FMD viruses (Bachrach, 1968).

The diluent used in the HA test, for both the antigen and the erythrocytes, was the phosphate-buffered saline solution of Dulbecco & Vogt (1954), pH 7·2, containing 0·9 mM Ca<sup>2+</sup>, 0·5 mM Mg<sup>2+</sup> and 0·1% crystallized bovine plasma albumin. Serial, twofold, 0·05 ml. dilutions of haemagglutinin in disposable micro-plates were each mixed with an equal volume of a 0·23% suspension of guinea-pig erythrocytes and then incubated at 37° C., for about 1 hr., until the cells settled. One unit of haemagglutinin was read as the highest dilution of the antigen giving a pattern of complete or almost complete agglutination.

#### *Haemagglutination-inhibition (HI) test*

Serum to be tested was diluted in three volumes of phosphate-buffered saline, pH 7·2, inactivated at 56° C. for 45 min. and absorbed with 1 volume of packed, washed guinea-pig erythrocytes for 1 hr. at 37° C. followed by overnight at 4° C. The dilution of the serum after absorption was taken to be 1/5. The absorbed serum was diluted serially, twofold, in 0·025 ml. volumes, starting at 1/10, and then 4 units of haemagglutinin in 0·025 ml. were added to each dilution. The resulting mixtures were incubated at 37° C. for 30 min., followed by the addition of 0·05 ml. of 0·23% erythrocyte suspension. After further incubation at 37° C. the HI titre was read as the highest dilution of the serum which completely inhibited 4 units of haemagglutinin.

#### *Neutralization test*

Assay of sera for neutralizing antibodies was carried out by the cell metabolic inhibition (colour) test (Martin & Chapman, 1961) using primary monolayers of

Table 1. *Haemagglutination-inhibition tests with type SAT 2 foot-and-mouth disease viruses and type-specific, reference, bovine convalescent antisera*

Bovine convalescent antiserum to virus	Haemagglutination-inhibiting antibody titre to viruses		Homologous neutralizing antibody titre
	SAT 2 Uga 6/70	SAT 2 Tan 5/68	
O Lombardy	< 10	< 10	181
A 119	< 10	< 10	2,000
C Noville	< 10	< 10	2,500
SAT 1 SA 13/61	< 10	< 10	209
SAT 2 Uga 6/70	256	128	256
SAT 3 Bec 1/65	< 10	< 10	400
Asia 1 Kemron	< 10	< 10	29,000

pig kidney cells and virus strains adapted to pig kidney cell cultures by serial passage. Neutralization titres were expressed as the reciprocal of the final dilution of serum present in the serum virus mixture at the 50% end-point estimated according to the method of Karber (1931).

In some tests, in an attempt to achieve greater accuracy, the sera were titrated against three separate doses of virus calculated to contain 10, 100 and 1000 TCD 50. A regression plot of serum titre versus actual dose employed in the test was used to determine the antibody titre against exactly 100 TCD 50 of virus.

## RESULTS

### *Viruses*

Type SAT 2 virus strains grown in BHK 21 cells show differences in the efficiency with which they cause haemagglutination (Booth & Pay, 1973) and the strains used in this study were no exception. Thus, with SAT 2 Uga 6/70 and SAT 2 Tan 5/68 virus antigen preparations, haemagglutinin titres were always about tenfold higher than titres of complement-fixing activity. With the SAT 2/3 Ken 3/57 virus, however, haemagglutinin activity was seldom detected even with highly concentrated antigen preparations and, when achieved, the ratio of haemagglutinin titre to complement-fixing antigen titre was only 0.06.

### *Type specificity of the HI test with convalescent bovine antisera*

It was shown previously in tests with hyperimmune guinea-pig sera to all seven FMD virus types that the haemagglutination by type SAT 2 viruses was inhibited only by homotypic antisera (Booth & Pay, 1973). The same was found to be true when reference, type-specific, convalescent bovine antisera were titrated against SAT 2 Uga 6/70 and SAT 2 Tan 5/68 haemagglutinins (Table 1).

### *Comparison of the HI and the serum neutralization tests*

*SAT 2 Uga 6/70.* A total of 52 serum samples from 14 cattle used in a field trial of a SAT 2 Uga 6/70 vaccine were titrated for homologous neutralizing and HI antibody activity. The results showed that both tests were equally specific for

Table 2. *The specificity of the haemagglutination-inhibition test and the serum neutralization test with SAT 2 Uga 6/70 virus and cattle sera*

		SAT 2 Uga 6/70 virus haemagglutination-inhibiting antibody activity		
		Number of sera negative	Number of sera positive	Total (%)
SAT 2 Uga 6/70 virus neutralizing antibody activity	Number of sera positive	1	38	39 (75)
	Number of sera negative	13	0	13 (25)
	Total (%)	14 (27)	38 (73)	52 (100)

Table 3. *Geometric mean neutralizing (N) and haemagglutination-inhibiting (HI) antibody titres in cattle antisera to type SAT 2 virus strains*

Antisera to virus strains	Uga 6/70		Tan 5/68		Ken 3/57	
	N	HI	N	HI	N	HI
Uga 6/70	182	72	ND	ND	ND	ND
Tan 5/68	41	67	56	60	ND	ND
Ken 3/57	63	57	ND	ND	1905	ND

ND, Not done.

detecting virus antibodies, as 51 (98%) of the sera were recorded as being correspondingly either positive or negative (Table 2). Discordant results were obtained with only one serum sample which demonstrated a neutralizing titre of 1/32 but no detectable HI activity. In this particular case, however, the neutralizing activity was probably non-specific since the serum sample had been obtained immediately before vaccination and, moreover, a second serum sample taken from the same animal on the sixth day after vaccination gave completely negative results in both tests.

A comparison of the HI and the neutralizing antibody titres recorded for individual sera showed that a good positive correlation existed between them ( $r = 0.932$ ,  $P < 0.001$ ; Fig. 1). Furthermore, the geometric mean antibody titres calculated for each set of data revealed that the neutralization test was 2.5 times more sensitive than the HI test (Table 3).

*SAT 2 Tan 5/68.* Twenty-one serum samples from seven cattle used in the potency testing of a SAT 2 Tan 5/68 virus vaccine were titrated for HI and neutralizing activity against the homologous virus strain. Both tests agreed completely in detecting antibody activity in only 14 of the sera and the titres obtained in each case were positively correlated ( $r = 0.916$ ,  $P < 0.001$ ; Fig. 2). Furthermore, the respective geometric mean titres showed that the two tests were of equal sensitivity (Table 3), in contrast to the findings with the SAT 2 Uga 6/70 virus.

Since the studies with SAT 2 Uga 6/70 virus and homologous cattle antisera had shown that the neutralization test was 2.5 times more sensitive than the HI test,

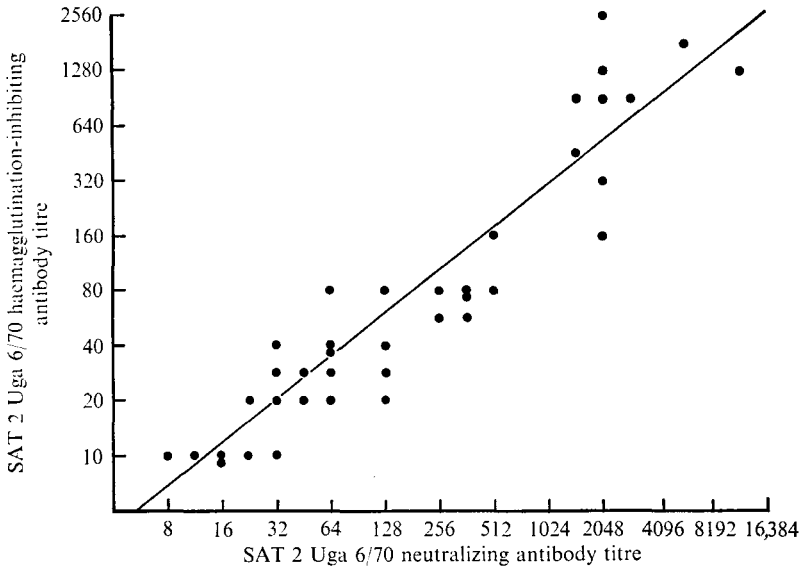


Fig. 1. Comparison of haemagglutination-inhibiting and serum neutralizing antibody titres to SAT 2 Uga 6/70 virus in homologous cattle antisera.

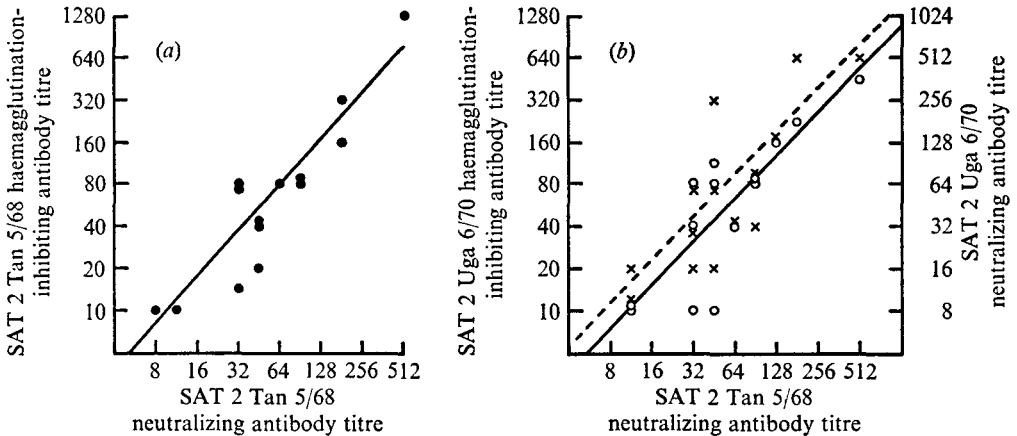


Fig. 2. (a) Comparison of haemagglutination-inhibiting and serum neutralizing antibody titres to SAT 2 Tan 5/68 virus in homologous cattle antisera. (b) Comparison of SAT 2 Uga 6/70 virus haemagglutination-inhibiting ( $\times$ , dashed regression line) and serum neutralizing ( $\circ$ , continuous regression line) titres, respectively, with titres of SAT 2 Tan 5/68 virus neutralizing activity in SAT 2 Tan 5/68 cattle sera.

it was of interest to find out whether a similar difference was demonstrated in tests with the same virus and the SAT 2 Tan 5/68 cattle sera. When the necessary titrations were done, it was evident that the results obtained were not significantly different from those recorded with the homologous SAT 2 Tan 5/68 virus antigen (Table 3; Fig. 2). In this set of tests, therefore, the neutralization and the HI tests were found to be equally sensitive for detecting antibodies reacting with SAT 2 Uga 6/70 virus.

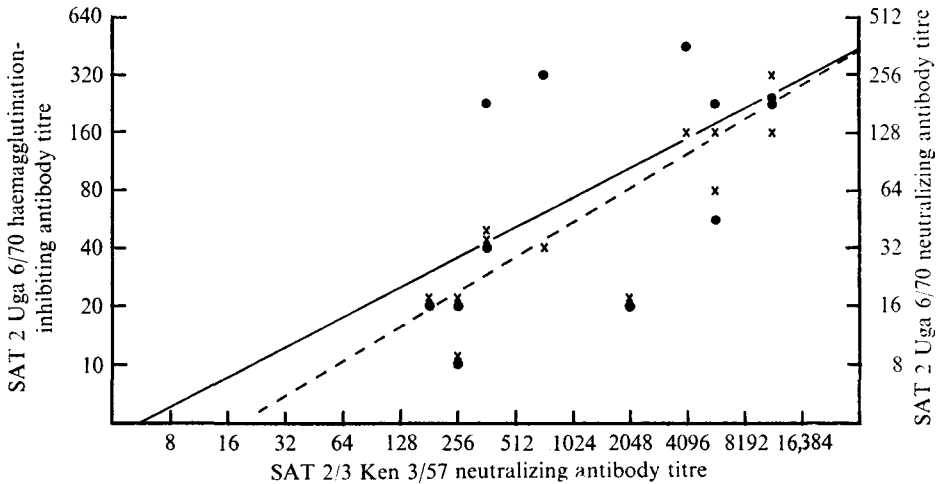


Fig. 3. Comparison of SAT 2 Uga 6/70 virus haemagglutination-inhibiting (x, dashed regression line) and serum neutralizing (●, continuous regression line) titres, respectively, with titres of SAT 2/3 Ken 3/57 virus neutralizing antibody activity in SAT 2/3 Ken 3/57 cattle sera.

*SAT 2/3 Ken 3/57.* As mentioned previously, haemagglutination by SAT 2/3 Ken 3/57 virus preparations was seldom demonstrated. It was therefore of some importance to find out whether antibodies to this virus could be detected in HI tests with a highly effective haemagglutinating virus as antigen. Consequently, 21 serum samples from seven cattle used in a potency test on a SAT 2/3 Ken 3/57 vaccine were titrated for neutralizing activity to the homologous virus and also for both HI and neutralizing activity to the SAT 2 Uga 6/70 virus. Twelve of the serum samples gave positive results in all three tests, including the HI test, whereas a further seven sera contained no detectable antibody activity. The remaining two sera had no neutralizing or HI activity to the SAT 2 Uga 6/70 virus but demonstrated neutralizing activity towards the SAT 2/3 Ken 3/57 virus at dilutions of 1/64 and 1/181 respectively. Moreover, there was no evidence to suggest that the neutralizing activity was not specific.

With the twelve sera shown to contain virus-specific antibodies in all three tests, comparisons of the titres obtained revealed a good positive correlation between the SAT 2/3 Ken 3/57 neutralizing and the SAT 2 Uga 6/70 HI activity ( $r = 0.804$ ,  $P < 0.001$ ; Fig. 3) and a lesser, but nonetheless significant, correlation between the SAT 2/3 Ken 3/57 and the SAT 2 Uga 6/70 neutralizing antibody titres ( $r = 0.591$ ,  $P < 0.05$ ; Fig. 3). Furthermore, there was no obvious difference between the sensitivity of the neutralization and the HI tests with the SAT 2 Uga 6/70 virus as these gave geometric mean titres of 1/63 and 1/57 respectively (Table 3). Thus, although the SAT 2/3 Ken 3/57 virus demonstrated little in the way of haemagglutinin activity, it was nevertheless still capable of inducing high levels of HI antibody activity. Indeed, the proportion of HI to neutralizing antibody activity to the SAT 2 Uga 6/70 virus in the SAT 2/3 Ken 3/57 antisera was not altogether different from the proportion found in antisera against the SAT 2 Uga 6/70 virus itself.

It was evident that a clear serological difference existed between the SAT 2/3 Ken 3/57 and the SAT 2 Uga 6/70 viruses. Thus, the geometric mean neutralizing antibody titre to the SAT 2/3 Ken 3/57 virus (1/1905) was 30 to 33 times greater than the mean titres obtained from neutralization and HI tests with the SAT 2 Uga 6/70 virus (Table 3). This large difference in antibody titres to the two virus strains explained the earlier finding that two of the SAT 2/3 Ken 3/57 cattle sera with homologous neutralizing antibody titres of 1/64 and 1/181 contained no detectable neutralizing or HI activity to the SAT 2 Uga 6/70 virus, even at the lowest dilutions tested (1/8 and 1/10 respectively).

#### DISCUSSION

The worth of the direct HI test as a serological tool in virology has been well proved and need not be elaborated upon. In the case of the haemagglutinating type SAT 2 FMD virus strains used in this study the HI test with cattle sera is type-specific and is as effective as the serum neutralization test in discriminating between sera which are positive or negative for virus antibody activity. Moreover, the antibody titres obtained by both test methods are, for the most part, closely similar to one another even with heterologous antisera.

The HI test does suffer from one drawback which limits its general applicability compared with the neutralization test, that not all type SAT 2 virus strains are equally effective in causing haemagglutination. Thus, it has been almost impossible to demonstrate haemagglutination with the SAT 2/3 Ken 3/57 virus whereas both the SAT 2 Uga 6/70 and the SAT 2 Tan 5/68 viruses regularly produce high titres of haemagglutinin activity. Other virus strains producing intermediate degrees of haemagglutinin activity have been described (Booth & Pay, 1973). Nevertheless, despite this limitation it is evident that the HI test will find a useful application in serological studies with type SAT 2 FMD viruses, especially those strains which are highly effective at causing haemagglutination.

The authors wish to acknowledge the capable assistance of Miss Pauline Haskell and Mrs Anne Heald.

#### REFERENCES

- BACHRACH, H. L. (1968). Foot-and-mouth disease. *Annual Review of Microbiology* **22**, 201-44.
- BOOTH, J. C. & PAY, T. W. F. (1973). Haemagglutination by type SAT 2 foot-and-mouth disease viruses. *Journal of General Virology* **19**, 397-404.
- COWAN, K. M. & WAGNER, G. C. (1970). Immunochemical studies of foot-and-mouth disease. VIII. Detection and quantitation of antibodies by radial immunodiffusion. *Journal of Immunology* **105**, 557-66.
- DE SIMONE, F., LODETTI, E., PANINA, G. & NARDELLI, L. (1970). Immunological responses in cattle vaccinated against foot and mouth disease under field conditions: II, titration of neutralizing antibody by the microculture plaque reduction technique (MPRT) and indirect complement fixation (ICF). *Atti della Società italiana delle scienze veterinarie* **24**, 580-83.
- DULBECCO, R. & VOGT, M. (1954). Plaque formation and isolation of pure lines with poliomyelitis viruses. *Journal of Experimental Medicine* **99**, 167-82.
- HEDGER, R. S., CONDY, J. B. & GOLDING, S. M. (1972). Infection of some species of African wild life with foot-and-mouth disease virus. *Journal of Comparative Pathology* **82**, 455-61.

- KARBER, G. (1931). Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Archiv für experimentelle Pathologie und Pharmakologie* **162**, 480–83.
- LODETTI, E., DE SIMONE, F. & NARDELLI, L. (1972). Neutralization tests for foot-and-mouth disease, equine rhino-, porcine entero-, Aujeszky- and Rhinopneumonitis viruses. *Zentralblatt für Veterinärmedizin* **B19**, 848–57.
- MARTIN, W. B. & CHAPMAN, W. G. (1961). The tissue culture colour test for assaying the virus and neutralizing antibody of foot-and-mouth disease and its application to the measurement of immunity in cattle. *Research in Veterinary Science* **2**, 53–61.
- REDA, L. M. & WITTMANN, G. (1972). Die Verwendung von CrCl<sub>3</sub> zur Kopplung von Maul-und-Klauenseuche-Virus an Erythrozyten und deren Eignung für die passive Hämagglutination und Immunnämolyse. *Archiv für die gesamte Virusforschung* **38**, 205–15.
- TOKUDA, G. & WARRINGTON, R. E. (1970). Detection of foot-and-mouth disease virus antibodies: I, 'passive' hemagglutination test. *Applied Microbiology* **20**, 35–9.
- WAGNER, G. G., COWAN, K. M. & McVICAR, J. W. (1972). Screening of sera for antibodies to foot-and-mouth disease viral antigens by radial immunodiffusion. *Infection and Immunity* **5**, 227–31.
- WAGNER, G. G. & McVICAR, J. W. (1970). Foot-and-mouth disease virus antibodies: comparison of a tissue culture microneutralization test with the assay in suckling mice. *Applied Microbiology* **20**, 995–7.
- WARRINGTON, R. E. & KAWAKAMI, Y. (1972). Passive hemagglutination-inhibition test for typing foot-and-mouth disease virus. *Applied Microbiology* **23**, 98–103.