

DIFFERENTIAL DIAGNOSIS OF VESICULAR STOMATITIS AND FOOT-AND-MOUTH DISEASE. EXAMINATION OF VIRUS SAMPLES FROM MEXICO WITH SPECIAL REFERENCE TO COMPLEMENT FIXATION

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(With 1 Figure in the Text)

In an earlier paper the author (1948) drew attention to the usefulness of the complement fixation test in the differentiation between vesicular stomatitis and foot-and-mouth disease. In that paper, it was stressed that owing to the fact that vesicular stomatitis* has not been found to occur in this country, work had been done on only two 'stock' strains of the virus. Since then, two samples of infective material have been received from the Joint Mexico-American Foot-and-Mouth Disease Commission. Both samples were believed to contain the virus of vesicular stomatitis, since workers in Mexico had inoculated horses and cattle intradermally on the tongue, and observed the occurrence of vesicular lesions at the sites of inoculation in both species. It is the purpose of this paper to describe the tests made to confirm these tentative diagnoses and to determine the immunological type of virus in each case. The part played by complement fixation in obtaining the required information rapidly and accurately is emphasized.

It is unnecessary to discuss here the relative merits of the various other methods of differentiation between vesicular stomatitis and foot-and-mouth disease, since they have been reviewed by Galloway (1936). In the observations recorded here, as many of these methods as possible have been applied to one of the samples of virus concerned (MEX. 1). The description of technique and the results obtained illustrate objectively some of the points discussed in the previous publication on the subject by the writer (1948). The examination of the second virus sample (MEX. 2) was limited to complement-fixation

* Unfortunately there has been a tendency to use the term 'vesicular stomatitis' in referring to what would appear to be a number of different conditions of obscure etiology associated with inflammatory lesions of the tongues of cattle. In this paper vesicular stomatitis refers to the well-defined virus disease.

and cross-immunity tests, the results of which are summarized after the description of the examination of the first sample.

MATERIAL AND METHODS

Examination of strain MEX. 1

The information received from Mexico was that the material sent for examination was harvested on 13 November 1947, from a calf at Salto de los Salados, Aguascalientes, Mexico. The sample, consisting of portions of tongue epithelium, was received at Pirbright on 11 March 1948 in a buffered medium containing 50 % glycerine. The pH of the mixture was 7.5.

The two stock strains of vesicular stomatitis in use at this Institute and described in the earlier publication on this subject have now been identified definitely as the Ind. C (Indiana type) and NJM (New Jersey type) described by Shahan, Frank & Mott (1946). It is therefore proposed to refer to them by these abbreviations in this paper.

All inoculation of horses and cattle were made intradermally on the tongue.

Inoculation of cattle

Of two cattle inoculated with a suspension of the original material, one developed vesicular lesions on the tongue at 3 days after inoculation. The other, though in contact with the reactor in a loose box measuring 18 x 12 ft., remained healthy. It was subsequently found to be susceptible to intradermal inoculation, developing full vesicular lesions when reinoculated 20 days later with a suspension of epithelium from the third bovine passage of strain MEX. 1.

At the second passage, two cattle inoculated with material from the reactor to the original sample both

developed vesicular lesions in 24 hr., and one also developed a small lesion on the upper lip at 7 days after inoculation. No other evidence of secondary lesions was found in these animals.

Complement-fixation tests

Virus from the first bovine passage was tested for fixation of complement with stock vesicular stomatitis antisera of Indiana and New Jersey immunological types and stock foot-and-mouth disease antisera of Vallée O, Vallée A and Waldmann C types. The method for the preparation of these antisera in guinea-pigs was outlined by the author in the paper referred to above (1948) in which the technique of the test was described briefly. This test gave a clear-cut result indicating that strain MEX. 1 was of the Indiana type of vesicular stomatitis. A complement-fixation test was not possible with the original material as there was insufficient epithelium to prepare a suitable antigen and at the same time allow of inoculation of cattle. The speed with which a diagnosis could be made by complement fixation was therefore limited in this instance by the time necessary for development of lesions in the animals inoculated.

Further evidence on the immunological identity of strain MEX. 1 with strain Ind. C was provided by a test in which an antiserum to strain MEX. 1, prepared in guinea-pigs, was included. The cross-fixation between viruses (bovine epithelial suspensions) and antisera (guinea-pig) of strain MEX. 1, Ind. C and NJM is shown in Table 1. The antisera were all from

Table 1

Serum strain	Virus strain	Virus strain		
		Ind. C	MEX.1	NJM
Ind. C	Ind. C	0.57	0.61	0.06
	MEX. 1	0.24	0.33	0.04
	NJM	0.02	0.05	0.10

The figures in this table are the differences between the logarithms of the dose of complement necessary for 50% haemolysis in the presence of the paired reagents indicated and the dose necessary without reagents. Correction is applied for the control virus alone and serum alone.

convalescent as distinct from hyperimmunized guinea-pigs and this explains the relatively poor fixation with the New Jersey virus-antiserum mixture. The point which it is desired to illustrate, however, is that, comparing strains MEX. 1 and Ind. C., no greater degree of fixation occurred when the virus and antiserum were strictly homologous (of the same strain) than when they were homologous only in the sense of being of the same immunological type. Observations in this Institute on Mexican strains of the virus of foot-and-mouth disease (Brooksby, Galloway & Henderson, 1948) have demonstrated

antigenic differences between strains of virus within one immunological type by means of similar cross-complement-fixation tests. The present results indicate no such antigenic differences between strains MEX. 1 and Ind. C.

Inoculation of horses

Two horses were inoculated with a filtrate (0.55 μ Gradocol membrane) of a suspension of material from the first and second bovine passages of strain MEX. 1. Two days later both developed full vesicular lesions at the site of inoculation on the tongue. Though there was extension of these lesions over the dorsum of the tongue, there were no secondary lesions.

Particle size by ultrafiltration analysis

The first attempt at the estimation of the size of virus of strain MEX. 1 was shown, by the bacteriophage control, to be unsuccessful. The filter membrane which was expected to retain both strain MEX. 1 and the Staph. K. bacteriophage in fact held back neither. Presumably this was due to a defect in the membrane used. A description of this experiment is given here as it illustrates the filtration technique generally followed in this laboratory and shows the usefulness of including a bacteriophage of appropriate estimated size as a control.

First filtration experiment. Epithelium was collected from the three cattle reacting to the first and second passages of strain MEX. 1. From one animal, the material was taken on the day of the experiment, and from the other two it had been stored at -20° C. without preservative. Seventy-five ml. of a 1/30 suspension in Hartley's broth were prepared, including 5 ml. of a Staph. K. bacteriophage suspension. The suspension was centrifuged and then filtered through a sand-and-paper pulp filter. This filtrate was then taken through a Gradocol membrane of A.P.D. 0.55 μ using negative pressure. Three 17 ml. amounts of this filtrate were then pipetted on to membranes of A.P.D. 0.22, 0.14 and 0.11 μ held in positive pressure mounts. At from 10 to 15 lb./sq.in. positive pressure, filtration through these membranes was rapid. In the first 10 min. 10, 5 and 5 ml. respectively were filtered. These first aliquots of filtrate were discarded, and the second 6, 10 and 10 ml. used for the subsequent tests, which consisted of the inoculation of cattle and the titration of the bacteriophage by the plaque count method.

The tests of each filtrate may be summarized as follows:

550 m μ filtrate. Two cattle inoculated intradermally in the tongue with dilutions of filtrate, five sites with each dilution in each animal. Dilutions used: 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} . Virus titre: $10^{-3.5}$. Titre of bacteriophage: 3×10^5 plaques per ml.

Differential diagnosis of vesicular stomatitis

220 m μ filtrate. Two cattle inoculated with a series of dilutions similar to that used for the 550 filtrate. Virus titre $10^{-2.8}$. Titre of bacteriophage: 4.7×10^4 plaques per ml.

140 m μ filtrate. Two cattle inoculated at numerous sites on the tongue with undiluted filtrate. Both

tongue. Titre of bacteriophage: 5.5×10^2 plaques per ml.

As noted above, the result of this experiment was at once vitiated by the demonstration of Staph. K bacteriophage in the 110 m μ filtrate, in fairly high titre. Earlier experiments have shown the filtration

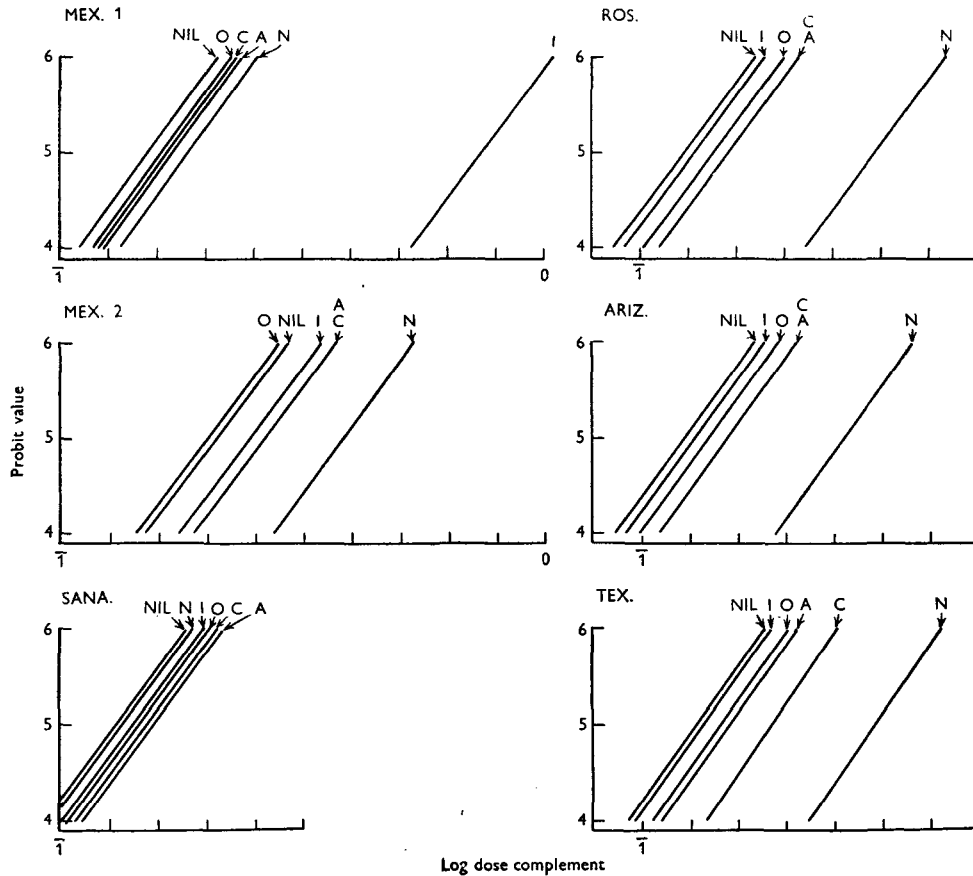


Fig. 1. Results of complement-fixation tests on six virus samples referred to in the text and addendum. In each case, a sloping line represents the titration of complement in the presence of the antigen ('unknown' virus) and a particular antiserum, probit percentage haemolysis having been plotted against log dose of complement. Fixation is shown by the degree to which a particular line is situated to the right of the 'NIL' control system. The stock antisera are indicated by the letters N and I for Vesicular Stomatitis, New Jersey and Indiana types respectively and O, A and C corresponding to the standard types of foot-and-mouth disease virus. Corrections have been applied for the effects of the various sera on complement. The results indicate that MEX. 1 virus is of the Indiana type of Vesicular Stomatitis, while MEX. 2, ROS., ARIZ. and TEX. are of the New Jersey type. In the case of SANA. no result has been obtained by the complement-fixation test, and it is of interest that guinea-pigs inoculated with this material failed to develop lesions.

reacted at 2 days with extensive vesicular lesions on the tongue. Titre of bacteriophage: 4.1×10^2 plaques per ml.

110 m μ filtrate. Two cattle inoculated at numerous sites on the tongue with undiluted filtrate. Both reacted, one at 1 and the other at 3 days after inoculation, with extensive vesicular lesions on the

end-point of this bacteriophage to be 110 m μ (Elford & Andrewes, 1932). The inference is that the 110 m μ membrane was defective, and evidence from this experiment on the particle size of Strain MEX. 1 is limited to the fact that it is not greater than that corresponding to membranes of APD 140 m μ .

Second filtration experiment. This experiment was

carried out on the same lines as that described above. Unfortunately, no further 110 m μ membranes were available, and a 120 m μ membrane was substituted. The 550 m μ filtrate, prepared as before, was therefore divided between 220, 140 and 120 m μ membranes. Owing to shortage of accommodation only two of the filtrates could be tested in cattle. The 120 m μ filtrate was inoculated at multiple sites in the tongue of two cattle and the 550 m μ filtrate was titrated in the same way as in the first experiment in a further two animals. A number of embryonated hen eggs, incubated for 8–10 days were available, and these were used for the detection of virus in dilutions of the various filtrates. The Staph. K bacteriophage included as a control of the filtration process was titrated by the plaque count method.

All manipulations were carried out on the day of preparation of the stock filtrate. Exposure of the filtrates to light was avoided as much as possible and after the completion of the filtration, filtrates and dilutions were kept at 4° C. until inoculated. The results of these observations are presented in Table 2.

Table 2

Filtrate through membrane of A.P.D. (m μ)	Titre of strain MEX. 1 virus in		Titre of Staph. K bacteriophage (plaques per ml.)
	Cattle	Developing eggs	
550	10 ^{-2.5}	10 ⁻⁵	1.7 × 10 ⁷
		(Not tested at 10 ⁻⁶)	
220	—	10 ⁻³	1.2 × 10 ⁶
140	—	10 ⁻¹	1.1 × 10 ⁶
120	No virus present (40 sites negative)	No virus present (survival of embryo in 4/4 eggs)	2.0 × 10 ⁴

Note. (1) The titres in eggs were based on only one egg per dilution in the series inoculated. The presence of vesicular stomatitis virus in the eggs reacting at the two highest dilutions was demonstrated by the development of vesicular lesions following inoculation of a suspension of the chorio-allantoic membranes into guinea-pigs. (2) The 'end-point' membrane of Staph. K bacteriophage is 110 m μ . In the above case only one or two plaques were present on each plate of a series of eight inoculated. The abrupt decrease in titre from 140 m μ to 120 m μ membrane filtrate indicates that the filtration end-point lies close to 120 m μ .

In the estimation of filtration end-points, it is best to construct a filtration curve showing the titre of filtrates through a series of membranes of decreasing porosity. This was not possible in the present instance, since the data from both cattle and eggs were insufficient for the purpose. In the second experiment, the titre of the stock filtrate, though lower than in the first, was high enough to make significant

the failure of the virus to pass the 120 m μ membrane, as shown by the negative result in both cattle and eggs. If the virus concerned were as small as that of foot-and-mouth disease there should be no decrease in titre of the magnitude suggested by the egg result until membranes of 70 m μ or less were reached. In all, the conclusion that the virus strain MEX. 1 has a particle size of 60–90 m μ appears justified. This is consistent with the size ascribed to vesicular stomatitis on the basis of experiments with guinea-pig passaged and egg-passaged virus, in which filtrates were tested in both guinea-pigs and eggs (Galloway & Elford, 1933, 1935) and with mouse passaged virus with filtrates tested in mice (Bauer & Cox, 1935).

Cross-immunity experiments in guinea-pigs

Groups of guinea-pigs convalescent 28 days from infection with the stock strains of vesicular stomatitis, Ind. C and NJM, and from strain MEX. 1 infection were inoculated intradermally in the plantar pads with suspensions of virulent epithelium from guinea-pig passages of these strains. The results, shown in Table 3, confirm the finding of the complement-fixation test that strain MEX. 1 is of Indiana type.

Table 3

Guinea-pigs convalescent from infection with strain	Strain inoculated			
	Ind. C	NJM	MEX. 1	MEX. 2
Ind. C	0 : 4	4 : 4	0 : 4	4 : 4
NJM	4 : 4	0 : 4	4 : 4	0 : 5
MEX. 1	0 : 4	4 : 4	0 : 4	3 : 3
Control normal guinea-pigs	8 : 8	8 : 8	8 : 8	7 : 7

Note. The numbers indicate the number of guinea-pigs showing lesions out of the number inoculated.

Examination of strain MEX. 2

According to information received from the 'Mexico-American Commission for the Eradication of Foot-and-Mouth Disease' this sample was from two cattle, nos. 153997 and 153998, which were infected with material from the first passage of a 'field' sample.

This field sample was collected by Dr R. R. Lindley in the Municipio of Jesus Carranza in the State of Coatzacoalcos, Mexico, from an animal showing 'lesions of suspected foot-and-mouth disease'. It was passaged during October 1948 by Drs Thompson and Manjarez, who found that lesions were produced after 48 hr. on the tongue of inoculated horses. This suggested that the virus of vesicular stomatitis was involved. The material sent to Pirbright was received on 15 November 1948. It was

preserved in a mixture similar to that used for strain MEX. 1.

Complement-fixation test. In this instance sufficient material was available, and a test was carried out on 16 November 1948. The antisera used included vesicular stomatitis, Indiana and New Jersey, and foot-and-mouth disease, Vallée O, Vallée A and Waldmann C types. As well as the stock Vallée A type antisera, there were included in the test antisera against two Mexican strains of foot-and-mouth disease virus, MP and M. 1. The result of the test was fixation of complement by strain MEX. 2 virus with the New Jersey antiserum and with no other.

Cross-immunity test. Strain MEX. 2 was passaged in guinea-pigs, and material of the third passage was inoculated into guinea-pigs immune to Ind. C, NJM and Mex. 1 infection. The result, shown in Table 3, clearly confirms the earlier observations in the complement-fixation test, and strain MEX. 2 can be assigned to the New Jersey type.

DISCUSSION

The outstanding feature of the observations recorded is that the promise of the complement-fixation test as a rapid method of differentiating between the viruses of vesicular stomatitis and foot-and-mouth disease has been fulfilled in the examination of two 'field' as distinct from 'experimental' strains of the virus of vesicular stomatitis. Unfortunately, owing to the small amount of material sent in the case of strain MEX. 1, the test could not in this instance be made on the original sample, and the rapidity with which the result could be obtained was limited by the time taken for passage in cattle. This preliminary passage was just as necessary to provide virus for the other methods of test which took several days to complete. Diagnosis by the complement-fixation test had therefore the advantage of the other methods by about 48 hr. With strain MEX. 2 more material was sent and a complement-fixation test was therefore possible with the original sample. This may have been accounted for by the fact that the 'field' sample had already been passaged in Mexico before being sent, which might make possible the collection

of a better sample of virus. The result of the complement-fixation test in this instance was obtained a few hours after the sample was unpacked in the laboratory. The result was later confirmed by the cross-immunity test in guinea-pigs which has been described.

The results obtained in the fractional filtration experiments confirmed that the particle size of strain MEX. 1 virus did not differ significantly from that of the accepted size value of the virus of vesicular stomatitis. The necessity of using cattle instead of eggs and guinea-pigs for determining the infectivity of filtrates in such an experiment merits some explanation. The objection as far as the use of the guinea-pig is concerned lies in the relatively lower susceptibility of the species as compared with cattle to foot-and-mouth disease virus of bovine origin. Similarly, it would be fallacious to assume that all strains of vesicular stomatitis virus can be propagated in developing hen eggs with equal ease.

Reference has been made to the examination in complement-fixation tests, of ten other virus samples from Mexico. These had all been tested (Brooksby *et al.* 1948) against stock foot-and-mouth disease (Vallée O, Vallée A and Waldmann C) and vesicular stomatitis (Indiana and New Jersey) guinea-pig type antisera. Positive results indicating that all ten samples contained the virus of foot-and-mouth disease and not that of vesicular stomatitis were obtained with Vallée A antiserum and with no other. All these tests, except one, were made with first passage virus material collected from cattle inoculated at Pirbright with the original samples. These passages were necessary, with the one exception, to secure enough material for the complement-fixation as well as other supplementary tests.

SUMMARY

The usefulness of the complement-fixation test in differential diagnosis between vesicular stomatitis and foot-and-mouth disease has been demonstrated on two 'field' specimens of virus from Mexico. Examples are given of the practical applications of the other methods for this differential diagnosis.

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ADDENDUM

Further evidence of the usefulness of the complement-fixation test has been obtained since the manuscript of this paper was prepared. Five additional samples of virus, from passages of 'field' strains, have been received from the Mexico-American Commission, and the Bureau of Animal Industry, U.S. Department of Agriculture, for confirmation of diagnosis and deter-

mination of immunological identity. The results of complement-fixation tests identified four of these samples, two from horses, one from cattle and one from guinea-pigs, as vesicular stomatitis, New Jersey type. No result was obtained from the test on the fifth, another sample from cattle, and an attempt to infect guinea-pigs with this material also failed.

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