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Strains of Virus of Foot-and-Mouth Disease Recovered from Outbreaks in Mexico. Identification.

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(Introduced by R. E. Shope.)

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These observations were made during the examination of samples of virus which were recovered from cattle during an epizootic in Mexico. It was reported that foot-and-mouth disease existed in Mexico in the last quarter of 1946 and now in June, 1948, fresh outbreaks of the disease are still occurring. Up to the present 13 separate samples of virus from Mexico have been examined at this Institute and as will be seen from the results recorded all of them could be classified in the main Vallée A immunological type group. After identification these virus samples were termed strains of the virus of foot-and-mouth disease. For the purpose of future reference a list is appended with the dates of collection and reported geographical origin:

- MP—Puebla, Dec. 23, 1946
- MPB—Puebla, Jan. 1, 1947
- MC—Camarones. Jan. 21
- MSL—San Lorenzo. Jan. 31
- M.1—Jalisco, Oct. 7
- M.2—Cuerrero, Oct. 11
- M.3—Toluca, Sept. 20
- M.4—Lerma, Sept. 30
- M.5—El Salto, Jalisco, Nov.
- M.6—El Bajo, Jalisco, Mar. 19, 1948
- M.7—Guzman, Apr. 12
- M.8—Zapotiltic, Apr. 13
- M.9—Zapotiltic, Apr. 13

So far the antigenic behavior of only 2 of the virus strains MP (23.12.46) and M.1 (7.10.47) have been investigated in some detail but further work is proceeding on the Mexican strains of virus and the results will be reported later.

These studies on antigenicity which are reported in other papers of the present series include complement fixation, serum neutralization and vaccination experiments.

Identification of infective agent and gen-

eral immunological type classification.

1. *Animal Species Susceptibility.*

(a) Inoculation of cattle.

The respective inoculation of suspensions of the thirteen samples of material received intradermally into the tongues of cattle has produced in every case a generalized vesicular disease resembling, in its general characteristics, foot-and-mouth disease.

(b) Inoculation of horses and donkeys. Suspensions of some of the samples of material received were inoculated intradermally into the tongues of horses and donkeys as follows:

- M.1—2 horses and 2 donkeys
- M.2—2 horses
- M.3—2 horses
- M.4—2 donkeys
- MP—2 donkeys

and contrary to what might have happened if the virus of vesicular stomatitis had been involved, no lesions were observed in any instance.

(c) Inoculation of swine. Two swine were inoculated intramuscularly with a suspension of the MP strain of virus after passage in cattle, one showed a vesicular lesion on the snout on the third day and on the feet on the fourth day and the other failed to develop lesions. A third pig inoculated by scarification of the snout and intradermally into the tongue with a suspension of the MP strain of virus showed no lesions.

(d) Inoculation of guinea-pigs. It was possible to infect guinea-pigs with suspensions of virus samples MP, MPB, MC, MSL, M.1 and the 9 strains to M.9 by inoculation intradermally into the pads of the hind feet and a generalized vesicular disease developed after adaptation to this species. Reference is made later to typing tests in guinea-pigs.

2. *Complement Fixation Tests.* These

have been made with stock type foot-and-mouth disease and vesicular stomatitis guinea-pig antisera. Tests involving the use of "Indiana" and "New Jersey" vesicular stomatitis stock type antisera were made with the 10 "virus samples" MP, and M.1 to M.9 and all yielded negative results. These vesicular stomatitis type antisera had been tested against their respective agents and had been proved type specific.¹

Complement fixation tests involving the use of Vallée A, Vallée O and Waldmann C stock type foot-and-mouth disease antisera were carried out with all the 13 virus samples and positive results were obtained in every case, except one (MPB), with the Vallée A antiserum and not with the Vallée O and Waldmann C antisera.

The complement fixation tests were carried out in most cases with first passage material, collected from the tongue lesions of inoculated cattle, as antigen but in one instance at least (MP) a positive result was recorded when a suspension of the original material sent, in 50% glycerine phosphate at pH 7.6, was employed as antigen. Negative results were recorded in the tests with MPB both on the original sample and on the first passage cattle material but the matter was not pursued further as typing tests in guinea-pigs and in cattle had already indicated that MPB could be placed in the Vallée A type group of the virus of foot-and-mouth disease.

3. *Differential Filtration.* Strains MP and M.1 after adaptation to guinea-pigs were examined respectively by fractional filtration through graded "gradocol" (Elford) collodion membranes and the end-point of 25 m μ enables a size value of 8 to 12 m μ to be attributed to both. This is the size value previously recorded for all strains of foot-and-mouth disease virus examined. The size of the virus of vesicular stomatitis is about 8 times as great, viz. 70-100 m μ .

4. *Inoculation into developing hen eggs.* It was shown that MP virus would not survive when inoculated on the chorio-allantoic membranes of developing hen eggs incubated

at temperatures of 35°C to 39°C (*cf* the virus of vesicular stomatitis which can be propagated in eggs under these conditions). Strain M.1 behaved similarly.

5. *General Immunological typing in guinea-pigs.* Five of the Mexican virus strains MP, MPB, MC, MSL and M.1 after adaptation to guinea-pigs were examined in cross immunity tests with standard type guinea-pig strains 1 (Vallée O) GB (Vallée A) and GC (Waldmann C) and as a result all 5 were classified as of the Vallée A type of the virus of foot-and-mouth disease.

6. *General immunological typing in cattle.* In all 28 cattle recovered from infection with known Vallée A type strains of the virus of foot-and-mouth disease have been inoculated with 8 of the virus strains under discussion viz. MP (8), MPB (4), MC (4), MSL (4), M.1 (2), M.2 (2), M.3 (2) and M.4 (2). With the first 5 strains the inoculations were made intramuscularly and none of the 22 cattle inoculated showed any lesions; 14 of these cattle were recovered from infection with the Pirbright Vallée A type stock cattle strain 119 and 8 had recovered from infection with an Argentine (A. Cor) Vallée A type cattle strain of the virus of foot-and-mouth disease. On the other hand 9 control susceptible cattle similarly inoculated respectively with the different virus samples MP (2), MPB (2), MC (1), MSL (2) and M.1 (2) all developed a generalized infection. With the remaining 3 virus strains M.2, M.3 and M.4 cattle recovered from infection with Vallée A type strain 119 were inoculated, 2 cattle for each strain, intradermally into the tongue with suspensions and although local reactions at the site of inoculation were recorded in 5 out of the 6 animals, no secondary lesions followed on the feet and elsewhere whereas a generalized disease involving these sites was observed on all of 6 control susceptible cattle similarly inoculated. In addition it may be mentioned that the relationship as regards the main group immunological typing (Vallée A) of the Mexican virus strains was further illustrated by the fact that of 8 cattle recovered from infection with the Mexican strain MP and subsequently inoculated either intramuscularly (M.1) or

¹ Brooksby, J. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 254.

intradermally into the tongue (M.2, M.3 and M.4) with 4 other Mexican strains of the virus, none developed any lesions.

Furthermore a limited number of tests were made by inoculating Mexican strains of virus into cattle recovered from infection with Vallée O and Waldmann C type strains of the virus of foot-and-mouth disease and in all of these a generalized foot-and-mouth disease infection resulted.

On perusal of the accumulated evidence it will be seen that none of it would suggest that any infective agent other than the virus of foot-and-mouth disease was present in any of the 13 samples of material collected from

cattle in Mexico and forwarded to Pirbright for examination. Moreover each of the 13 strains could be clearly classified in the main Vallée A immunological type group of the virus under discussion. The examination of these Mexican strains of virus involved inoculation of cattle, horses, donkeys, swine, guinea-pigs and developing hen eggs, fractional filtration experiments, complement fixation tests and cross immunity typing tests in guinea-pigs and cattle against stock type strains of the virus of foot-and-mouth disease and it was therefore much more exhaustive than that usually made of "unknown" samples of virus.

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Strains of Virus of Foot-and-Mouth Disease Recovered from Outbreaks in Mexico. Pathogenicity and Invasiveness.

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A report on the identification of samples of virus received from Mexico has been made.¹

Before referring to the general effects of the Mexican strains of virus in cattle exposed to infection by inoculation, by contact with diseased cattle, or by the nasal instillation of virus, something should be said about the animals employed in these experiments.

Foot-and-mouth disease is not endemic in Great Britain but is introduced at irregular intervals, outbreaks occurring sporadically. Since the policy of control is by "stamping-out," that is, elimination of infected and "in-contact" animals by slaughter and burning (or under the exigencies of war, by burial) along with the rigorous application of other disinfection and "stand-still" orders, it is possible only to purchase cattle with a "clean" history as regards foot-and-mouth disease. The cattle used in these experiments

were Devon steers between 1½ and 2 years old, in good condition and weighing between 600-800 lbs, and they could be considered as relatively "standard."

1. *Intradermal inoculation into the tongue.*

In all 296 cattle have been inoculated with centrifuged suspensions or stock filtrates of the 13 strains of Mexican virus. 184 were inoculated with MP virus, 80 with M.1 virus, and the remaining 32 with the other 11 strains. 293 of these 296 reacted well, with local lesions at the sites of inoculation and generalized or secondary lesions at some or all of the usual sites of predilection, feet, and lips, in all cases except 2. These 2 were control animals inoculated with filtrates in an experiment described elsewhere.²

There were 3 cattle which failed to show lesions at the sites of inoculation. These had been inoculated with similar virus material

¹ Brooksby, J. B., Henderson, W. M., and Galloway, Ian A., *Proc. Soc. Exp. Biol. and Med.*, 1948, **69**, 64.

² Henderson, W. M., Galloway, Ian A., and Brooksby, J. B., *Proc. Soc. Exp. Biol. and Med.*, 1948, **69**, 77.