

STRAINS OF THE VIRUS OF FOOT-AND-MOUTH DISEASE SHOWING NATURAL ADAPTATION TO SWINE

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(With 10 Figures in the Text)

The occurrence of strains of the virus of foot-and-mouth disease showing more or less marked species adaptation, or preferential infectivity for one of the host species commonly attacked, has been mentioned by earlier observers. In Schleswig-Holstein in 1927, for example, there occurred an epidemic of the disease in which swine were affected to the almost total exclusion of cattle (Waldmann, 1929; Waldmann & Trautwein, 1929). The strain or strains of virus involved were of type Vallée O, and in the laboratory the disease could be transmitted to cattle by inoculation. The examination of three similar strains of virus from field outbreaks in Britain has already been recorded from this Institute (Andrews, Eccles, Hole, Polding, Longley, Hamilton & Graham, 1937). These three strains were readily transmissible from pig to pig, but infection of cattle could be produced only by inoculation, and even then only irregularly. The experiments on exposure of cattle to contact infection were limited to one bovine exposed to infected swine and two exposed to infected cattle. In both cases the recipient animals failed to develop the disease.

Opportunities are not frequent for the study of possibly species-adapted strains of foot-and-mouth disease. Since, however, the question of the nature of the pathogenic agent involved may arise when frequent outbreaks of vesicular disease in swine occur with no sign of spread to cattle, investigations may be necessary for differential diagnosis. It was with this point in mind that the present series of observations was made.

Early in 1944 there was a sharp increase in the number of samples of foot-and-mouth disease virus from swine sent to this Research Institute from outbreaks in the field in Britain. This is strikingly evident from the data recorded in Table 1. Routine investigations for the determination of the immunological type of the virus involved were carried out on thirty of the porcine strains out of a total of 113 received in 1944, 1945 and 1946. No unexpected results were obtained in these experiments, and it therefore appeared to be unlikely that an infective agent other than the virus of foot-and-mouth disease was involved in the outbreaks, since it seemed absurd to postulate a new pathogen with the characteristics of an already known immunological type of the virus. Since only approximately 1 in 4 of the strains were examined, the objection may be raised that a more complete study of the several materials received might have revealed the presence of another virus. The samples studied, however, were selected from all parts of the country, and it

is believed that if another agent had been involved, even to a slight extent, it would have been detected. In defence of the limited typing of field strains it may be stated that at this time the complement-fixation test had not been fully developed and that 176 specimens of virus were received in the three-year period; since the typing of strains in guinea-pigs requires anything from thirty to fifty or more guinea-pigs, it was considered to be uneconomic to study more than the sixty-three (bovine and porcine) actually investigated.

Table 1

Year	Total outbreaks (no. of premises affected)	Total samples of virus (received at Institute)	Species of animal from which samples sent had been collected			
			Bovine	Porcine	Bovine and porcine in same outbreak	Ovine
1940	336	42	40	1	0	1
1941	448	53	52	0	1	0
1942	675	51	43	7	1	0
1943	20	7	5	2	0	0
1944	180	83	31	51	0	1
1945	129	61	6	49	5	1
1946	59	32	15	13	4	0
1947	96	33	24	7	1	1
1948	14	11	8	1	2	0

- N.B. (1) In 1941, on the premises involved in the primary outbreaks there were in 34 cases pigs present, but on only 4 occasions were they diseased at the time of slaughter.
- (2) In 1942, in 36 cases pigs were present, but disease was observed in only 9. In both these years, swill fed to pigs was frequently implicated as a possible cause of the outbreaks.
- (3) In 1944, on the premises from which bovine material was received, there were in 24 cases swine present, and among these cases there were only 6 in which the swine became affected. On the premises from which porcine material was sent, there were in 15 instances cattle, but the disease was in no case found in this species.

Some of the data recorded in Table 1 were obtained from the official records of the Animal Health Division of the Ministry of Agriculture and Fisheries to whom my thanks are due.

The evidence from type determination is sufficiently strong to make it unnecessary, in our opinion, to carry out investigations such as are usually planned for the differential diagnosis of foot-and-mouth disease from the other recognized vesicular diseases of cattle, sheep and pigs. Such tests as those of animal susceptibility, cultivation in developing eggs, estimation of particle size by determination of the filtration end-point, and determination of pH stability of the virus are of great service when such general information as had been obtained in the present series is lacking. In the series of outbreaks under consideration it seemed more important to find out how a particular strain of virus, recovered from an outbreak in swine, would behave experimentally under controlled conditions of contact exposure. Observations on three strains of the virus of foot-and-mouth disease are recorded in the next section. Differences in degree of species adaptation such as are shown between these strains may explain some of the anomalies in the behaviour of the virus under field conditions.

STRAIN 643

This strain was received on 12 June 1946 from an isolated outbreak confined to one pig at Bathampton. An opportunity to examine the virus did not arise till August of the same year, when a suspension of the original porcine epithelium was inoculated intralingually by the intradermal route into a steer. Vesicular lesions were present at the site of inoculation within 22 hr, and at 46 hr. there were lesions on two feet.

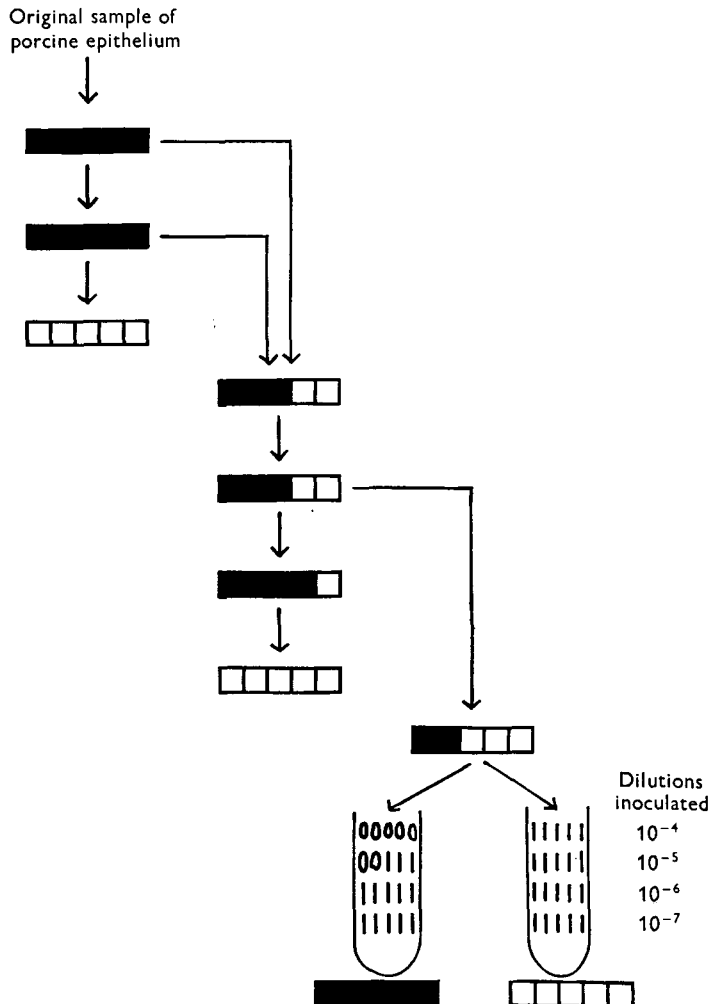


Fig. 1. Passage of strain 643 in cattle. The serial passages, indicated by arrows were all by intradermal inoculation of the tongue. $\square \square \square \square$, indicates one steer. Occurrence of lesions is shown by blackening, the square on the left for the tongue, and the others for the four feet. **O**, Lesion at inoculated site on tongue. **I**, Inoculated site with no lesion.

On 11 September the first passage in swine was made by inoculating two pigs intradermally into the tongue and snout, with a suspension of the original material. These pigs showed well-developed tongue lesions at 1 and 2 days respectively after inoculation and lesions on all four feet by the fifth day.

instance with a strain of virus passaged by direct tongue to tongue inoculation and it was particularly striking.

At the titration of fourth passage material only one of the two cattle used reacted, although the non-reacting animal received virus in a tenfold higher concentration than that which produced 'takes' at two out of five sites in the other animal.

The contrast between the results of the series of passages in cattle and that in swine, shown in Fig. 2, is well marked. No difficulty was experienced in maintaining the strain in pigs and the lesions produced were very severe. No distinction could be drawn between the clinical picture and that produced by other strains associated with severe foot-and-mouth disease in swine.

Infection following exposure by contact. On five occasions healthy cattle were in contact with infected cattle, and in only one instance did infection take place. The recipient animals in all but one of these tests had been inoculated previously intradermally into the tongue with virus and had failed to react. In all cases the dose of virus inoculated, as estimated in other animals, was very small, and from our experience it would be justifiable to ignore the inoculations in considering that infection failed to pass from donor to recipient in the contact exposure tests.

Contact infection took place readily from one pig to two healthy pigs, and when two infected and two healthy pigs were placed in contact with two bullocks the two healthy pigs developed lesions at 4 and 6 days respectively from the beginning of contact, while the bullocks remained healthy for 14 days. These results are summarized in Fig. 3, where the duration of contact with donor infected animals is given.

The animals involved were run together in loose-boxes 12 ft. square, except in the case of (1) the test with three bullocks, when a box 12 by 18 ft. was used and (2) the test with cattle and pigs when two boxes 12 ft. square, with a communicating door, were used.

Some details of the contact exposure test with pigs and cattle are justified to demonstrate the opportunity for infection. The two bullocks used each weighed between 5 and 6 cwt. and the four pigs approximately 100 lb. The animals were somewhat suspicious of each other for the first few hours, but settled down quite amicably later, the pigs rapidly learning to keep clear of the bullocks' hind feet. After 3 or 4 days, it was not unusual for the bullocks to be found one in each box with the pigs moving freely back and forth between the boxes. The bullocks were fed from two hay-racks on the walls, and the pigs from troughs. Within a week, the bullocks began to eat the pig food, so they were then fed first with hay and an attendant remained in the box while the pigs fed. A small amount of straw was given as litter for the pigs.

Titration and vaccination. The repeated failures to infect cattle by contact, and the 'sluggish' behaviour of the strain on passage by intradermal inoculation into the tongue, suggested that the pathogenicity of the strain for cattle might be lowered sufficiently by passage in pigs to allow of its use as a vaccine. The serial passage in pigs shown in Fig. 2 was carried out for this purpose, and material was prepared and an estimate of its virus content obtained by inoculation of dilutions

into cattle and pigs. The titration in cattle was carried out by the method of Henderson (1949), inoculating twenty sites on the tongue of two cattle with the serial dilutions. Six pigs were inoculated, 1:10⁻², 2:10⁻³, 3:10⁻⁴ and 4:10⁻⁵, with dilutions of the same series. The result, shown diagrammatically in Fig. 4, suggests at least a hundredfold difference between the titres of the same suspension in cattle and pigs, and provides more evidence of the adaptation to the latter species.

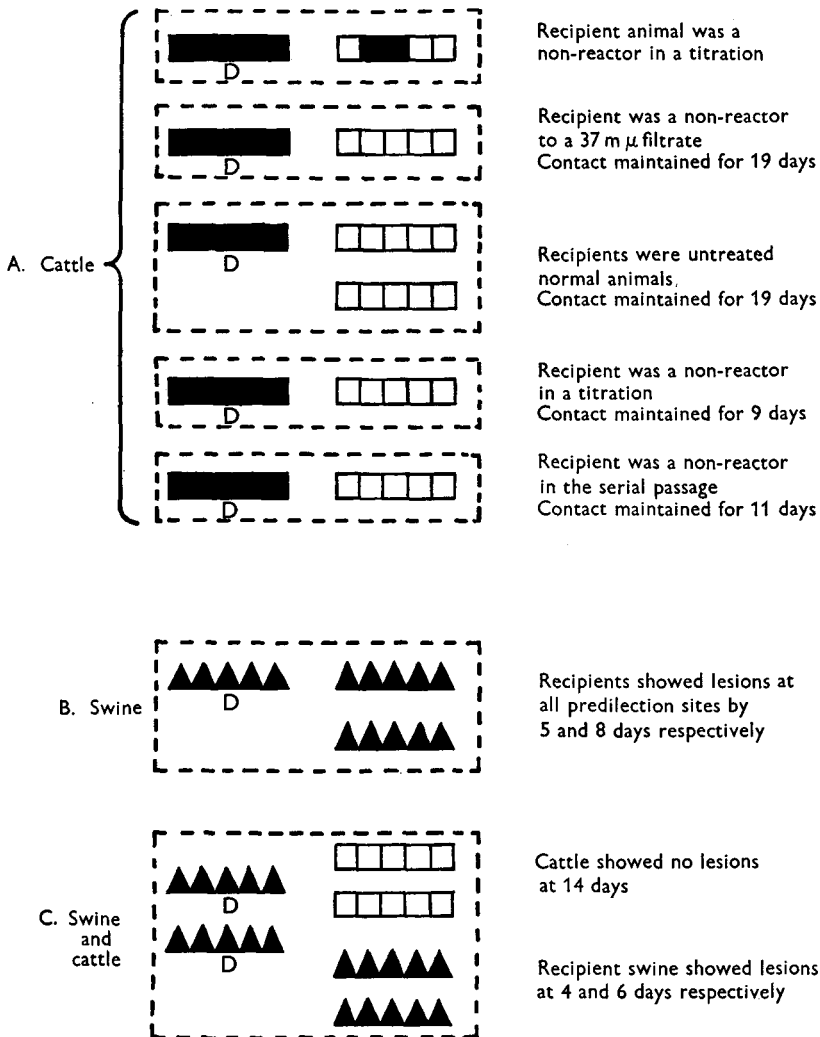


Fig. 3. Exposure to infection by contact. Strain 643. Lesions are indicated as in Figs. 1 and 2. Dotted lines surround the group in contact. D, indicates donor infected animal.

Four healthy cattle were given 10 ml. of the original suspension subcutaneously. Four days later one was found with lesions on all four feet and it was removed from the group. The remaining three remained healthy (Fig. 5) and at 14 days were exposed to contact infection from an animal infected with strain ASJ (Vallée O type). A healthy animal was included as a control, and at 4 days two animals

recovered from strain 643 infection were also included. The result of this contact exposure test is shown in Fig. 6.

It is clear that the dose of active virus of strain 643 which was high enough to infect one of the 'vaccinated' animals by the subcutaneous route had failed to stimulate the defence mechanism of the others sufficiently to allow them to withstand contact infection successfully. The fact that the recovered animals withstood the disease, although they were exposed to successive 'waves' of infection with the

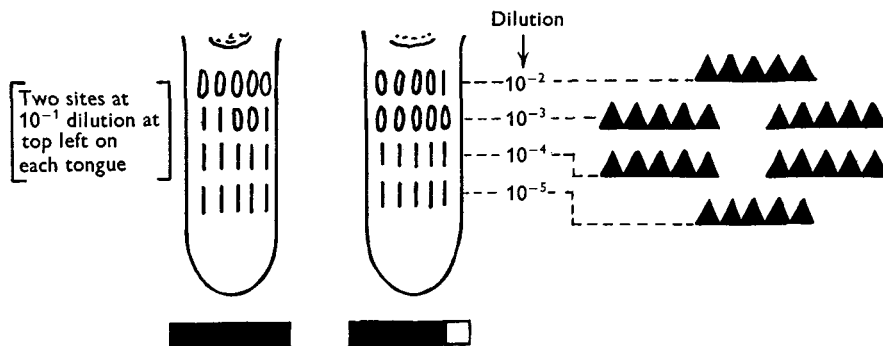


Fig. 4. Comparative titration in cattle and swine of a suspension of epithelium of the 9th porcine passage. Strain 643. Lesions indicated as in Figs. 1 and 2.

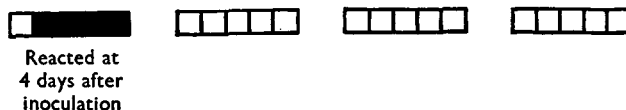


Fig. 5. Attempted vaccination of cattle using the same suspension as titrated in animals shown in Fig. 4. Four cattle were given 10 ml. suspension subcutaneously.

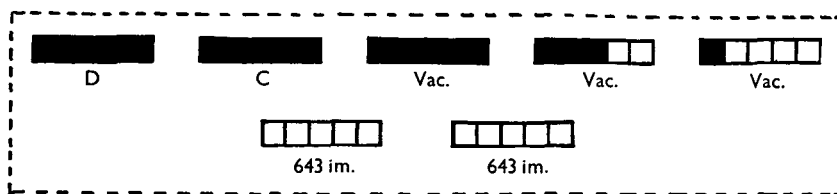


Fig. 6. Exposure of 'vaccinated' cattle to infection with strain ASJ. D=infected donor animal. C=untreated control. Vac.=vaccinated animals. 643 im.=Animals recovered from strain 643 infection. Both remained healthy. The experiment was discontinued after 19 days.

development of the lesions in the control and 'vaccinated' animals provides a check on the typing of strain 643 as Vallée O. Exposure of the animals to contact infection with the homologous (643) strain rather than with the heterologous (ASJ) strain of the same type would have been preferable if the former had given evidence of spreading power in cattle. This failure of strain 643 to protect against strain ASJ infection might be due to slight differences in antigenic constitution between them, but the use of active virus as a vaccine could only be justified if the immunity conferred were of a degree strong enough to overcome minor antigenic variations of strains of virus within one type.

Filtration. Observations on the particle size of the virus of foot-and-mouth disease based on filtration studies have hitherto been made only on material from guinea-pigs. Two experiments were carried out with strain 643, one using bovine and the other porcine material. In general, the technique followed was that described by Galloway & Elford (1931).

A suspension of virulent epithelium was prepared in Hartley's digest broth, and after preliminary clarification by centrifugation and filtration through a sand-and-paper pulp filter it was passed through a series of graded collodion membranes ('Gradocol'). In both cases, virus could be detected in the 37 m μ filtrate but not in the 25 m μ filtrate, when these were inoculated into animals of the species from which the original material had been harvested. This is consistent with earlier results on the virus of foot-and-mouth disease in guinea-pig material, and furnishes corroboration of the earlier conclusion that strain 643 is indeed foot-and-mouth disease.

STRAIN 672

This strain was from a group of outbreaks in Middlesex and Berkshire in February 1947. The disease occurred mainly in large piggeries where the animals were fed on London hotel swill. Through movements of infected pigs, and subsequent journeying of vehicles in which they had been conveyed, pigs became infected on

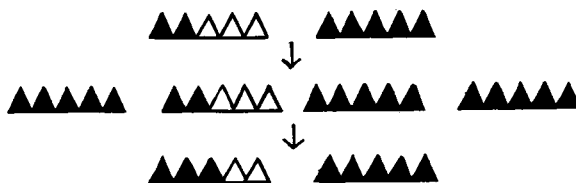


Fig. 7. Passage of strain 672 in swine. Symbols as in Fig. 2.

five premises at which cattle were housed. In spite of this and in spite of the fact that various groups of cattle were conveyed in the vehicles mentioned during the period of the outbreak, only on one farm was a single bovine affected, and here lesions were confined to the tongue.

Since this one case of infection in a bovine had occurred under natural conditions it was of interest to determine how the species adaptation of this strain compared with that of strain 643, which, as has been shown, was proved infective for cattle only with considerable difficulty.

Study of strain 672 was deferred owing to limitations of space until June 1948, when two passages in pigs were made from the original porcine epithelium. Further serial passage was not possible till January 1949, when the strain was revived from the first porcine passage in two pigs from which epithelium was collected and inoculated in a further two to give the third porcine passage. The reactions of these pigs are recorded in Fig. 7.

A suspension of third porcine passage material was used in the following tests.

Titration in cattle and pigs. The titration in cattle of the 1/10 suspension of porcine epithelium by the same method as used for strain 643 gave the value $10^{-2.7}$.

The titre in pigs was approximately the same, as can be seen from the reactions shown in Fig. 8.

Infection following exposure by contact. Two healthy cattle were placed in a loose-box 12 × 18 ft. with the cattle reacting in the virus titration experiment referred to above. Both 'recipients' reacted, one at 4 and the other at 10 days.

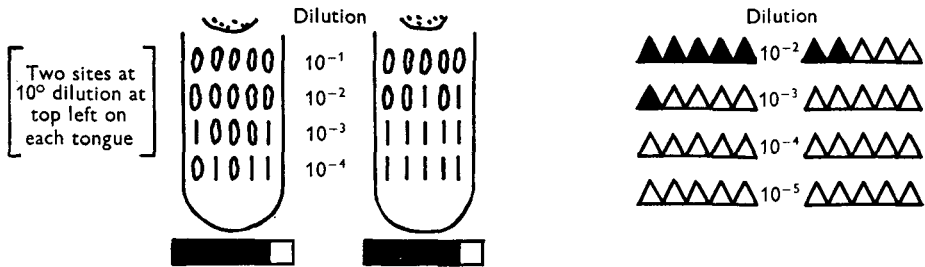


Fig. 8. Comparative titration in cattle and swine of a suspension of epithelium of the 3rd porcine passage of strain 672.

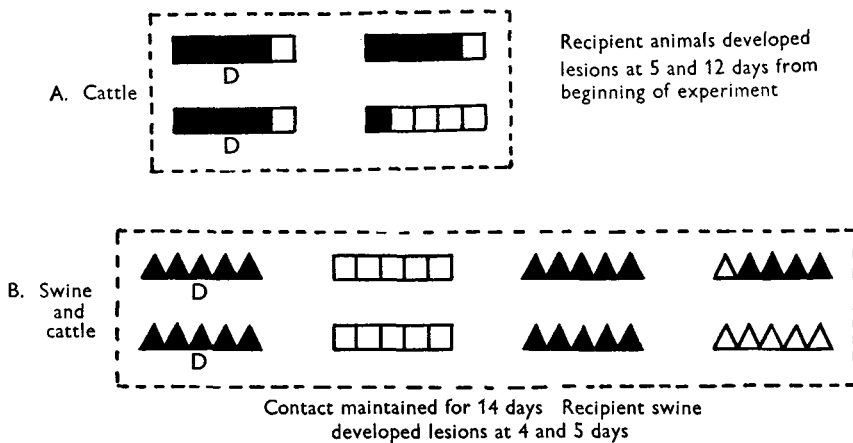


Fig. 9. Exposure to infection by contact. Strain 672. D, indicates infected donor animal.

In another experiment two pigs reacting after inoculation of a stock virus suspension were used as donors to two healthy cattle and four healthy pigs in a box 24 by 12 ft. communicating with one 12 ft. square. As can be seen from the diagram (Fig. 9), both cattle remained healthy while three out of four pigs became infected.

STRAIN ASJ

This strain was known from earlier work to be pathogenic for both pigs and cattle. It was received from Drs Schang and Rossi of the Argentine in 1944 and had been passaged frequently in cattle. Material from the 20th bovine passage at Pirbright, collected in November 1945, was passaged in two cattle in March 1949 and produced well-developed primary lesions. This material, inoculated into two

pigs, gave good reactions, and in a contact experiment similar to that described for strain 672 two cattle and three out of four pigs became infected from these two donor pigs (Fig. 10).

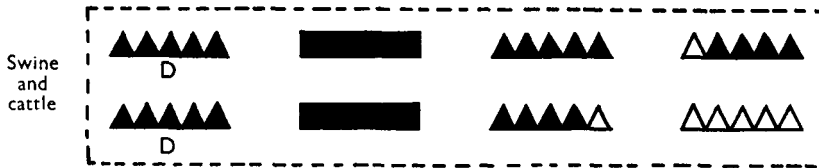


Fig. 10. Exposure to infection by contact. Strain ASJ. Recipient animals developed lesions at 5 and 6 days.

DISCUSSION

The various observations recorded are consistent with the view that virus strains 643 and 672 have a marked degree of species adaptation to swine, 643 being slightly the more strongly porcophilic. Under the conditions of experiment, strain ASJ would appear to infect pigs and cattle with equal readiness. This suggests that the failure of the other two strains to spread from pigs to cattle is probably due to factors implicit in the virus host relationship. With regard to the criteria on which species adaptation is to be judged, too few observations have been obtained on the relative titres of the strains in cattle and pigs. The titre of the virus suspensions concerned cannot be estimated so exactly in pigs as in cattle without the use of an uneconomic number of animals. The individual pig furnishes but one observation of the infectivity of dilutions, whereas the bovine tongue can be used for twenty observations. The 'titre' in pigs is therefore but a rough index of infectivity. In the case of strain 643 the titre in pigs is higher than in cattle, which result conforms to expectations. On the other hand, strain 672 appeared to be of approximately equal titre in the two species. Whether or no these results are due to the inadequacy of the titration in pigs is of little moment when it is obviously not possible to improve this titration at reasonable cost.

The difficulty experienced in passaging strain 643 in cattle was most striking. Such difficulty would probably not have been encountered with strain 672, as judged by the fact that contact infection appeared to take place readily from bovine to bovine with this strain. What would have been infective doses of strain 643 were inoculated in four cases into cattle by the intradermal route without producing the disease.

It would appear that an attempt to transmit the disease from pigs to cattle by contact infection is essential in the study of a supposedly species-adapted strain. Measurement of the degree of adaptation might be possible by repeated tests under controlled conditions. In the field it is usually impossible to have an exact picture of the opportunities for cross-infection, though much circumstantial evidence can be and is in fact accumulated. The state of the lesions in the affected pigs, besides many imponderables under the conditions of contact, will obviously influence the chances of infection of cattle. When, in continuous contact with infected pigs in our experiments, cattle failed to become infected, it is likely that

field infections of cattle with such strains as 643 and 672 will be few and far between. It is interesting to speculate on how frequently cattle came into contact with porcine virus in this country during the period 1944–6 and yet did not develop the disease.

The statement that the virus of foot-and-mouth disease is more 'virulent' for cattle after passage through swine is found frequently in the literature. Even if such a claim could ultimately be established for certain strains of virus by satisfactorily controlled experiment, it certainly could not be upheld in respect of virus strains exhibiting porcinophilic characters.

The effect of porcine-adapted strains on the epizootiology of the disease is difficult to assess. In Britain we are concerned with successive fresh importations or fresh invasions of the virus into a 'clean' stock. In the period 1944–6, the picture produced by these invading viruses may have been, as it were, a reflexion of the situation prevailing in an area oversea from which the viruses came, or, on the other hand, the porcine and bovine strains may have come from more widely separated localities. The geographical distribution of outbreaks in Britain during the period was entirely haphazard in respect of the species involved. It should be noted, too, that the incidence of initial outbreaks, or outbreaks not related to any earlier focus of infection, was relatively high. Since samples of virus from all initial outbreaks in the country are sent to this Institute, the figures in Table 1 relating to samples received are evidence on this point. The number of secondary outbreaks was, however, much smaller than in periods such as 1942, when there were fewer initial outbreaks but very many secondary ones. This is illustrated by the data on total outbreaks in Table 1. The variation in number of secondary, arising from initial, outbreaks has not been linked to the virulence or other properties of the strains concerned, since experimental evidence on this aspect is entirely lacking. In many cases the dissemination of infection will depend on such factors as the involvement of markets or dealers' premises, the proximity of infected stock to other animals, the rapidity with which the suspected case was reported and stand-still orders applied and so on. The porcine outbreaks of 1944–6 frequently did not involve more than the premises of the original infection. The cattle outbreaks were not widely different from those at times when the infection in the country was predominantly in bovines, though there were no major centres comparable with the Bath-Somerset group of 1942.

Another aspect of adaptation which must be considered is the epizootiological picture which might be produced by an adapted strain in a species other than its preferential host. A strain such as 672, infecting cattle, could produce mild lesions which would escape notice on casual inspection. The possibility of a mild infection of cattle, with little spread, being due to a porcine strain must always be taken into account, and passage in swine included in the investigation. Further, it must be emphasized that a mild clinical picture is not sufficient grounds for disregarding the disease, as even if the strain remains stable in its properties, infection of the species to which it is adapted might initiate an extensive outbreak.

SUMMARY

An account is given of the behaviour of two porcine field strains of the virus of foot-and-mouth disease, with evidence of their adaptation to swine, based on experiments on infection by contact.

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