Growth of foot-and-mouth disease virus in the upper respiratory tract of non-immunized, vaccinated, and recovered cattle after intranasal inoculation

By J. W. McVICAR and P. SUTMOLLER

Plum Island Animal Disease Center, Northeastern Region, Agricultural Research Service, U.S. Department of Agriculture, Greenport, L.I., New York 11944, *U.S.A.*

SUMMARY

Non-immunized, vaccinated, and recovered cattle were inoculated intranasally with various doses of foot-and-mouth disease virus. Samples of oesophageal-pharyngeal (OP) fluid were taken periodically for up to 7 days after inoculation and virus titres of these samples were plotted as pharyngeal virus growth curves.

In non-immunized cattle, the length of the lag period and of the growth period were inversely proportional to the dose of virus given. Maximum titres were observed when clinical signs were first detected. Three of the 10 cattle studied had virus growth rates that were lower than rates of others given the same dose of virus, and clinical signs appeared later than expected in these three cattle.

Cattle vaccinated with an inactivated virus oil-adjuvant vaccine had pharyngeal virus growth curves that were similar to those obtained from non-immunized cattle for 30 h. after inoculation. Titres of virus in OP fluid samples taken 2–7 days after inoculation were substantially lower in cattle with a high pre-exposure serum mouse protection index than titres from partly-immunized or non-immunized cattle.

Nine of 14 recovered cattle had detectable but reduced virus growth after intranasal inoculation with homologous virus. Five recovered cattle inoculated with heterologous virus reacted similarly to non-immunized animals.

INTRODUCTION

Foot-and-mouth disease (FMD) is most frequently transmitted by aerosol (Fogedby, Malmquist, Osteen & Johnson, 1960; Hyslop, 1965*b*; Sellers & Parker, 1969; Donaldson, Herniman, Parker & Sellers, 1970; Graves, McVicar, Sutmoller & Trautman, 1971). In cattle, virus can gain entrance through the upper or lower respiratory tracts or the eye, each of which has been shown to be susceptible

30-3

to experimental inoculation (Sutmoller, McVicar & Cottral, 1968; Eskildsen, 1968; Sutmoller & McVicar, 1973). Virus growth in the upper respiratory tract may precede the appearance of clinical signs for up to several days (Burrows, 1968; Graves *et al.* 1971).

Virus is isolated from samples of oesophageal-pharyngeal (OP) fluid, which is obtained by means of a cup probang inserted into the oropharynx and anterior oesophagus (Sutmoller, Cottral & McVicar, 1968). Pharyngeal virus growth curves produced by plotting the infectivity titres of OP fluid samples taken at regular intervals after virus exposure (Graves *et al.* 1971; McVicar, Graves & Sutmoller, 1971) can be used to study the infectious process.

Cattle vaccinated with inactivated FMD virus vaccines have become infected after upper respiratory exposure even to homologous virus subtypes (VanBekkum, Frenkel, Frederiks & Frenkel, 1959; Hyslop, 1965*a*; Burrows, 1966; Sutmoller *et al.* 1968; McVicar & Sutmoller, 1969). For a study of early growth of FMD virus in immunized cattle, steers were vaccinated and inoculated intranasally after varying periods of time, and pharyngeal virus growth curves were obtained.

Finally, cattle previously infected with FMD virus were re-exposed to either the homologous or a heterologous virus subtype, and pharyngeal virus growth curves were plotted.

MATERIALS AND METHODS

Cattle

Grade Hereford steers approximately 18 months old were housed in isolation units as described elsewhere (Callis & Cottral, 1968).

Virus

FMD virus, subtype O_1, strain CANEFA*-2 was used after seven passages in primary bovine kidney (BK) cell cultures.

Virus inoculation

Virus in 2·5 ml. of tissue culture medium was slowly instilled into the ventral part of each nasal passage through a 12 cm. length of latex tubing attached to a 5 ml. syringe. During inoculation, the head was raised so that the nose was on a level with the poll.

Storage of samples

Samples of OP fluid, heparinized blood, and serum were held at $-20°$ C. until tested.

Virus assay

OP fluid and blood samples were tested for the presence of virus by placing 0·2 ml. on primary BK cell cultures in 4 oz. prescription bottles (Bachrach *et al.*

* Commission Asesora National para la Eradicacion de Fiebra Aftosa.

1962). After 1 hr. at 37° C., the cultures were overlayered with 10 ml. of Hanks' balanced salt solution with 0·5 % lactalbumin hydrolysate (HLH), and incubation continued at 37° C. If cytopathic effect (CPE) was not observed at 24 and 48 hr. in cultures inoculated with samples from vaccinated or recovered cattle, the fluids were changed. Samples negative for CPE at 72 hr. were considered not to contain FMD virus. Positive samples were assayed in secondary BK cultures under gum tragacanth overlay (Mirchamsy & Rapp, 1968); titres were recorded as plaque forming units (p.f.u.) per ml.

Vaccine

Virus grown in baby hamster kidney cell cultures (BHK-21) was concentrated tenfold by precipitation with polyethylene glycol (Wagner, Card & Cowan, 1970) and inactivated for 24 hr. at 37° C. with 0·05 % acetylethyleneimine. The inactivated preparation was emulsified with an equal volume of adjuvant consisting of 9 parts of mineral oil and 1 part of mannide mono-oleate. A 2 ml. dose of this vaccine was given subcutaneously.

Serum assay

Suckling mice were used in mouse protection tests (Cunha, Baptista, Serrao & Torturella, 1957). Virus was titrated in untreated mice and in mice that 2 hr. before had been given subcutaneously 0·1 ml. of the heat-inactivated serum to be tested. Titres were expressed as the $\log_{10}$ LD50/ml., and the mouse protection index (MPI) was calculated as the difference between virus titres in untreated and serum-treated mice.

Plaque reduction neutralization (PRN) titres were expressed as the $\log_{10}$ of the reciprocal of the sample dilution that caused a 73 % reduction in plaques in secondary BK cell cultures (McVicar, Sutmoller & Andersen, 1974).

EXPERIMENTS AND RESULTS

Pharyngeal virus clearance

Two cattle each were inoculated with 10^6, 10^7, or 10^8 p.f.u. of virus. A cup probang was inserted in the oesophagus before inoculation and withdrawn immediately afterwards, and the volume of OP fluid obtained was measured. Additional OP fluid samples were taken at 1, 5, and 10 min. after inoculation and at 10 min. intervals until $1\frac{1}{2}$ hr. after inoculation.

Approximately 10 % of the inoculated virus infectivity was recovered from the anterior oesophagus immediately after inoculation. The titres of OP fluid samples taken subsequently declined at a rate of approximately 2·0 $\log_{10}$ p.f.u./ml./hr. The rate of decline slowed somewhat at 1 hr. after inoculation, but titres continued to fall for an additional 30 min.

Susceptible cattle

Eleven cattle were inoculated with doses of virus ranging from 10^3 to 10^7 p.f.u. Samples of OP fluid were taken with a cup probang every 20 min. until 2 hr.,
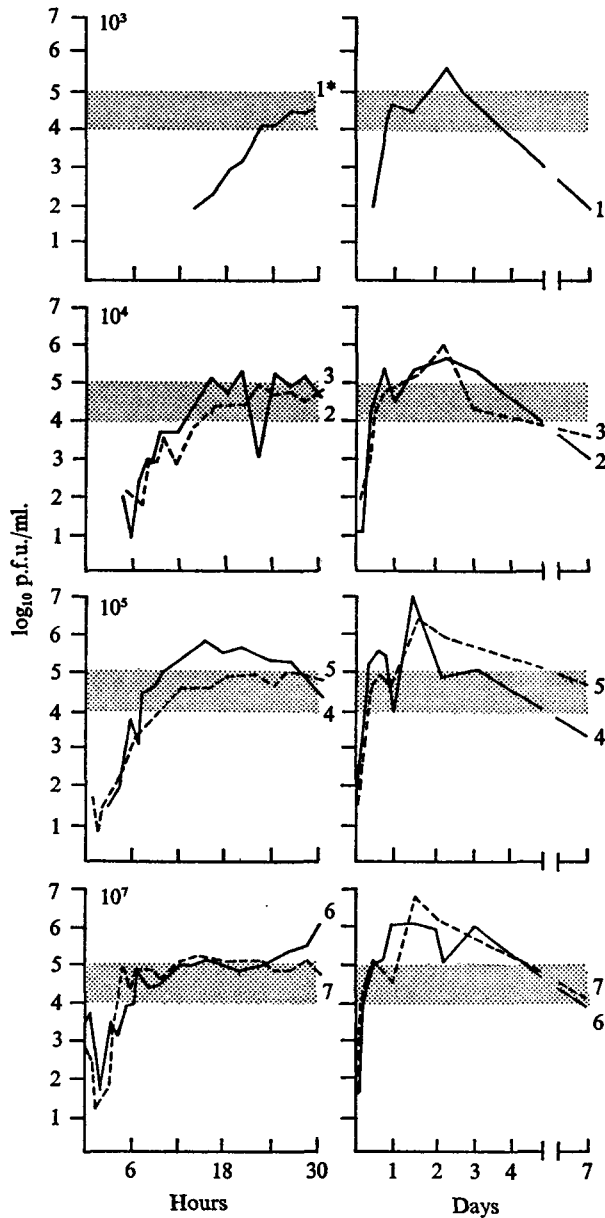
Fig. 1. Pharyngeal virus growth curves of non-immunized cattle after intranasal inoculation with different doses of foot-and-mouth disease virus type O. * = Steer number.

every hour from 3 to 10 hr., and then every 2 hr. from 12 to 30 hr. after inoculation. Heparinized blood samples were taken every 6 hr. for the first 30 hr. except for two steers (Steers 6 and 7) that were sampled more extensively. In these two, an indwelling polyethylene catheter was inserted into the jugular vein; and starting at 12 hr., blood samples were taken every 30 min. up to 30 hr., every hour up to 36 hr., and every 2 hr. up to 48 hr. after inoculation. Additional OP fluid
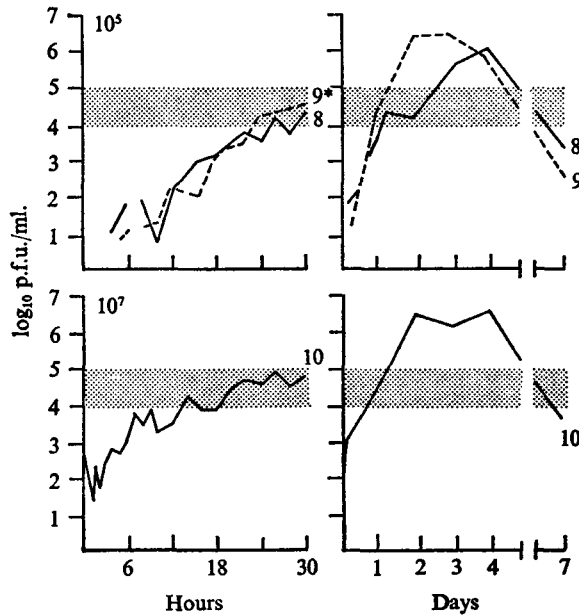
Fig. 2. Pharyngeal virus growth curves of non-immunized cattle showing a low apparent growth rate after intranasal inoculation with different doses of foot-and-mouth disease virus type O. * = Steer number.
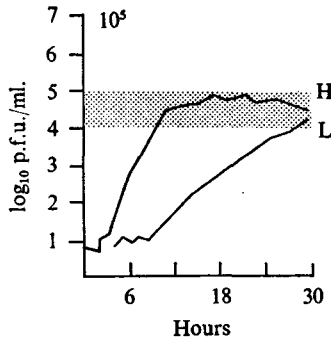

Fig. 3. Comparison of high (H) and low (L) apparent pharyngeal virus growth rates in cattle given 10^5 p.f.u. of foot-and-mouth disease virus type O intranasally.

samples were taken from these two steers every 2 hr. from 30 hr. up to 48 hr. Blood and OP fluid samples were taken from all cattle at 2, 3, 4, and 7 days after inoculation.

Ten of the 11 cattle inoculated became infected and developed clinical signs of FMD. Virus was not recovered from one of the two cattle given 10^3 p.f.u. of virus, and this steer remained free from FMD.

Pharyngeal virus growth curves of Steers 1 to 7 are presented in Fig. 1. Inoculum virus was present in the 20 min. samples of Steers 5, 6, and 7, given 10^5 or 10^7 p.f.u. of virus. Subsequently, titres declined at the expected rate as the virus was cleared from the pharynx. The interval between inoculation and the first sustained

Table 1. *Observations on non-immunized cattle after the intranasal inoculation of various doses of foot-and-mouth disease virus Type* O

Steer no.	Virus dose (p.f.u.)*	Growth rate	Lag period (HPI)†	First viraemia detected (HPI)†	First disease sign‡
1	10^3	High	14	48	FMD (3)
2, 3	10^4	High	6, 5	72, 48	FMD (3), FMD (3)
4, 5	10^5	High	3, 2	30, 30	FMD (2), FMD (2)
6, 7	10^7	High	2, 2	16, 22	FMD (2), FMD (2)
8, 9	10^5	Low	6, 4	48, 48	FMD (4), F (3)
10	10^7	Low	2	48	F, T (3)

* p.f.u. = plaque forming units.
† HPI = hours after inoculation.
‡ FMD = generalized foot-and-mouth disease; F = fever; T = tongue, ( ) = days after inoculation.

increase in virus titre of the OP fluid will be referred to as the lag period. In these seven cattle, the lag period varied from 2 to 14 hr. and became longer as the inoculum dose was decreased.

A growth period followed, after which virus titres levelled off at a plateau, usually between 10^4 and 10^5 p.f.u./ml. The curves were not smooth but rather made up of a series of steps or peaks approximately 6 hr. apart; apparently a number of such peaks were required before the virus titres consistently exceeded 10^4 p.f.u./ml. Maximum titres at 2 or 3 days after inoculation coincided with the first observation of clinical signs of FMD. At this time, there were unruptured vesicles, fever (rectal temperature $\geqslant 103 \cdot 0^\circ$ F.), and viraemia. During the subsequent disease period, virus titres in the OP fluid declined so that most were below 10^4 p.f.u./ml. by 7 days. At this time, virus was no longer detectable in the blood, oral lesions were healing but still very much in evidence, and foot lesions were severe.

The early pharyngeal virus growth curves of Steers 8, 9, and 10 given 10^5 or 10^7 p.f.u. of virus (Fig. 2) were quite different from those just described. Virus was found only sporadically in samples from Steers 8 and 9 taken up to 10 hr. after inoculation. The apparent growth rate was substantially lower in all three steers than in other cattle given similar doses of virus. Again, there was evidence of repeated 6 hr. peaks. In order to show the differences in growth rates more clearly, mean titres were separately calculated for Steers 8 and 9 and for Steers 4 and 5, which also had been given 10^5 p.f.u. of virus. Three-point moving averages were calculated, and the smoothed curves are presented in Fig. 3. Note that virus titres in the OP fluid eventually all reached approximately the same level.

Observations on the 10 susceptible cattle inoculated intranasally are summarized in Table 1. Viraemia was detected relatively late in the cattle with the lower virus growth rate, and clinical signs appeared later than in other cattle given the same dose of virus.
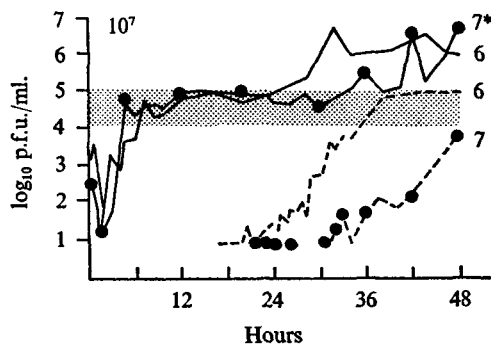
Fig. 4. Pharyngeal virus growth curves (●—●) and viraemia titres (● – – – ●) of non-immunized cattle after intranasal inoculation with foot-and-mouth disease virus type O. * = Steer number.

In tests to establish the relation between pharyngeal growth and viraemia, Steers 6 and 7, given 10^7 p.f.u. of virus, were sampled frequently up to 48 hr. (Fig. 4). Virus titres of the OP fluid peaked at 36–42 hr. and also 6–12 hr. later when clinical signs were first detected. In Steer 6, viraemia was first detected at 16 hr. During the next 8 hr., virus was not detected consistently; but at 24 hr., the virus titre of the blood began a relatively steady rise and increased for 14 hr. before levelling off at approximately 10^5 p.f.u./ml. In Steer 7, sporadic virus detection started at 22 hr., and the virus titre of the blood began a sustained rise only at 34 hr. The titre was still increasing at 48 hr. when blood sampling was stopped.

Vaccinated cattle

One of seven vaccinated cattle was inoculated with virus 140 days after vaccination. The rest were inoculated as pairs at 28, 40, and 60 days. The single steer was given 10^7 p.f.u. of virus; one of each pair was given 10^5 p.f.u. and the other was given 10^7. Pre-inoculation OP fluid and serum samples were taken for antibody assay. Thereafter, OP fluid samples were taken every 20 min. until 2 hr. and then every 2 hr. until 24–30 hr. after inoculation with the exception of Steers 11, 13, and 14 which were not sampled at 8, 10, 12, or 30 hr. Clinical observations were made, and blood and OP fluid samples were taken from all of the cattle at 2, 3, 4, and 7 days after inoculation.

Pre-inoculation OP fluid samples did not contain specific neutralizing activity. The results of mouse protection and PRN tests of pre-exposure serum samples as well as a summary of observations made after virus inoculation are presented in Table 2. As expected, a relation existed between the pre-exposure serum antibody titres and the subsequent development of clinical signs. The result of both antibody assays showed that all cattle had serum antibodies against FMD virus; however, the MPI allowed the cattle to be easily divided into three groups. Cattle with a pre-exposure serum MPI under 2·0 developed fever or lesions, or both, after virus exposure. One of the two cattle with an MPI between 2·0 and 4·0 had a single foot lesion 7 days after exposure whereas the two steers

Table 2. *Pre-exposure status of cattle immunized with an inactivated, oil adjuvant foot-and-mouth disease virus vaccine and observations after intranasal inoculation with the homologous virus*

Steer no.	Pre-exposure serum		Lag period (HPI)‡	Growth rate	Clinical signs§
	MPI*	PRN†			
11	0·7	1·8	2	High	Fev (2) F (4)
12	1·2	2·1	2	High	T, F (6)
13	1·3	1·9	2	High	Fev (3)
14	3·1	2·7	2	High	None
15	3·8	2·1	2	High	F (7)
16	⩾ 5·5	2·8	10	Low	None
17	⩾ 5·5	2·3	8	Low	None

* MPI = Mouse protection index.
† PRN = plaque reduction neutralization titre.
‡ HPI = hours after inoculation.
§ Fev = fever; F = foot lesions; T = tongue lesion; ( ) = days after inoculation.
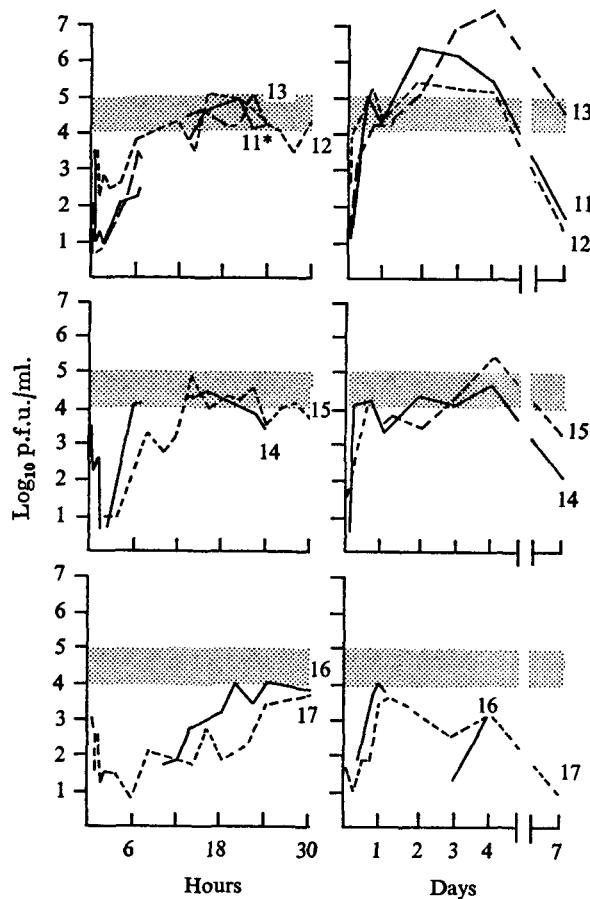

Fig. 5. Pharyngeal virus growth curves of vaccinated cattle after intranasal inoculation of the homologous foot-and-mouth disease virus type O. * = Steer number.
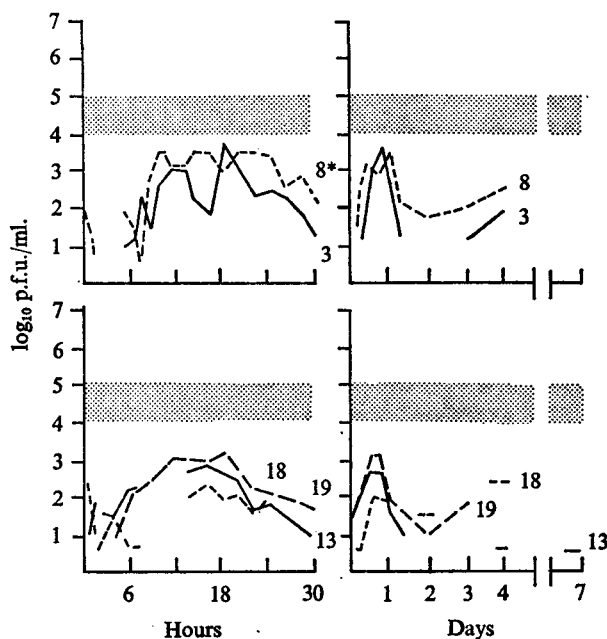
Fig. 6. Pharyngeal virus growth curves of recovered cattle after intranasal inoculation with the homologous foot-and-mouth disease virus. * = Steer number.

with an MPI over 5·0 remained clinically normal. Clinical reactions appeared later and were never as severe as those seen in non-vaccinated cattle exposed to the same virus strain. Fever seldom lasted more than 1 day, and lesions were erosive and few in number. Viraemia was not detected in any of the vaccinated cattle. Differences in pharyngeal virus growth could not be related to length of time from vaccination to virus exposure, and differences between animals given the two doses of virus were minimal.

Pharyngeal virus growth curves of the vaccinated cattle (Steers 11 to 17) are presented in Fig. 5. Inoculum virus was detected in the 20 min OP fluid samples of each steer, but thereafter the virus was cleared at the same rate as had been observed for non-immunized cattle. Up to 30 hr. after inoculation, the curves of all seven vaccinated steers resembled those of non-immunized cattle given the same virus dose. The curves of Steers 16 and 17 indicated a low virus growth rate (Figs. 2, 3). The later curves of Steers 11 to 13 were also similar to those of non-immunized cattle, but lower titres were observed in Steers 14 to 17, which had higher pre-exposure serum MPI's. Contrary to observations with susceptible cattle, maximum titres did not always coincide with the appearance of clinical signs. In five of the seven steers, peak titres were recorded at 4 days after inoculation.

Recovered cattle-homologous virus exposure

Nine of the previously described 17 cattle and 5 cattle from another experiment were inoculated with 10^7 p.f.u. of the homologous virus strain 5–39 weeks after

Table 3. *Pre-exposure status of cattle recovered from infection with foot-and-mouth disease virus of subtype* O_8, A_{20}, *and* C_3 *and observations after intranasal inoculation with virus of subtype* O_1

Steer no.	Virus subtype	Carrier*	Lag period (HPI)†	Growth rate	Clinical signs‡
23	O_8	−	4	High	FMD (3)
24	O_8	−	4	High	Fev, G (3)
25	A_{20}	−	2	High	FMD (3)
26	A_{20}	+	6	High	FMD (3)
27	C_3	+	6	Low	Fev (3) F (6)

* Virus in oesophageal-pharyngeal fluid at time of re-exposure.
† HPI = hours after inoculation.
‡ FMD = generalized foot-and-mouth disease; Fev = fever; G = gum lesion; F = foot lesion; ( ) = days after inoculation.

their initial inoculation. Samples of blood and OP fluid were taken before inoculation and, except where noted, according to the same schedule as that for the vaccinated cattle. Three of the cattle had virus in the OP fluid at the time of the second inoculation and may be considered as carriers. The PRN titres of the pre-exposure sera ranged from 3·0 to 3·8, and each of these sera had an MPI of 5·0 or greater. No neutralizing activity was detected in the OP fluid samples from five of the cattle. The PRN titres of the OP fluid from the other nine cattle ranged from 0·8 to 1·9. After inoculation, virus was not isolated from four of these nine cattle and was isolated only sporadically from the other five. There was, on the other hand, substantial virus growth in the five steers that had no detectable neutralizing activity in the OP fluid at the time of the second inoculation (Fig. 6).

Titres of the 20 min. OP fluid samples were approximately $1·0 \log_{10}$ p.f.u./ml. below those of non-immunized or vaccinated cattle given the same virus dose. From then on, however, titres in the early samples dropped at the same rate as had been seen in the other cattle. Lag periods ranged from 2 to 5 hr., and titres were highest about 12 hr. later. Steers 13 and 18 were not sampled between 6 and 14 hr., but all titres were declining by 18–20 hr. after inoculation. The curves of Steers 3 and 8 consisted of a series of peaks, each of which lasted for approximately 6 hr. When virus was present in a 4 day sample, it was always at a higher titre than in the 2, 3, or 7 day samples from the same animal. Trichlorotrifluoroethane (TTE) treatment of OP fluid samples taken up to 30 hr. after inoculation did not result in an increase in titre; but in later samples, the treatment led to either a substantial increase in titre over that of the untreated sample or to the detection of virus when none had been detected in the untreated sample.

Recovered cattle-heterologous virus exposure

Five cattle, 6–10 weeks after infection with one of three subtypes of FMD virus (O_8, A_{20} or C_3), were inoculated with 10^5 or 10^7 p.f.u. of virus subtype O_1. Samples of serum and OP fluid were taken before inoculation and at the same
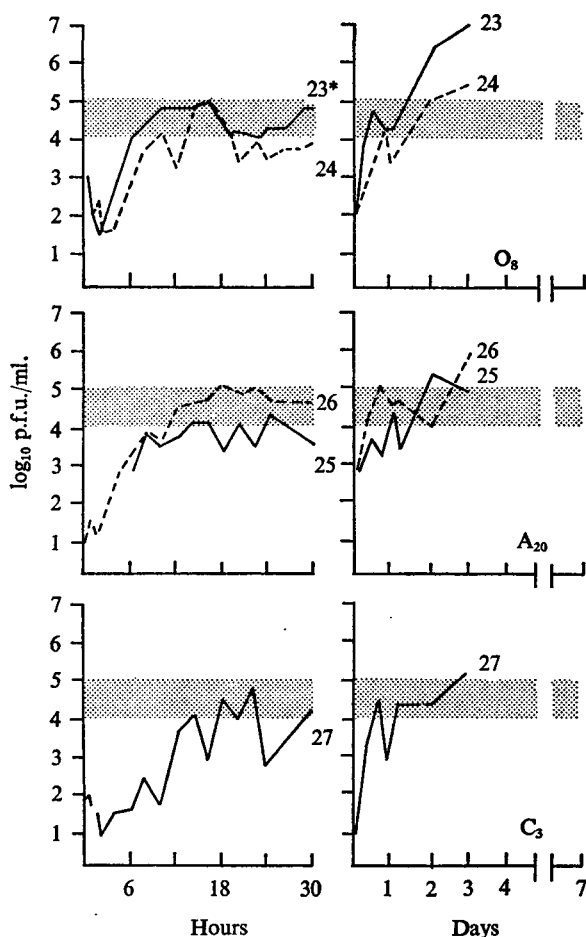
Fig. 7. Pharyngeal virus growth curves of recovered cattle after intranasal inoculation with a heterologous type or subtype of foot-and-mouth disease virus. The subtype of the initial infection is noted on the figure. The second inoculation was with FMD virus subtype O_1.

times as in previous groups of cattle with the exception that all sampling was stopped at 3 days after inoculation. For each steer, the pre-exposure serum MPI was greater than 5·0 when tested against the homologous virus and less than 1·0 against the O_1 virus. The pre-exposure sera of steers previously infected with O_8 virus neutralized O_1 virus slightly, but no similar activity was detected in the other sera nor in any of the OP fluid samples. A summary of the pre-exposure status of each steer and of observations made after inoculation is presented in Table 3. All five cattle had clinical signs at 3 days and developed severe disease. The growth curves presented in Fig. 7 are essentially identical with those of non-immunized cattle given similar doses of the same virus sub-type. Four of the five curves showed clear evidence of the 6 hr. virus peaks seen with other cattle. Steer 27 had a low growth rate and a somewhat delayed clinical syndrome.

DISCUSSION

Approximately 10% of the infectivity of virus inoculated intranasally in non-immunized cattle was immediately recoverable from the throat. Thus, a considerable part was either inactivated, neutralized, or retained within the upper respiratory tract. In this instance, because the time from inoculation to removal of the probang never exceeded 1 min., it would not be expected that much of the virus was inactivated. Furthermore, it is unlikely that the OP fluid would contain specific neutralizing activity because these six cattle had had no previous exposure to FMD virus. Therefore, a substantial amount of the inoculated virus was retained within the upper respiratory tract, probably either by becoming entrapped in the mucus or by being adsorbed to cells.

The titre of virus in succeeding OP fluid samples dropped rapidly, probably because of mechanical removal by the muco-ciliary blanket. This mechanical clearance is also the means by which the virus was carried into the oropharynx, where it became accessible to collection by the probang. Indeed, mucus, combined with saliva and occasionally with traces of rumen fluid and bits of feed, makes up the OP fluid sample. VanBekkum, Straver, Bool & Frenkel (1966) obtained mucus samples from FMD-infected cattle by applying strips of filter paper directly to the pharyngeal mucosa. Virus titres of these samples were sufficiently higher than those of OP fluid samples taken concurrently to suggest that most of the virus was in the mucus itself.

The length of the lag period varied from 2 to 14 hr. in susceptible cattle and was inversely related to the dose of virus inoculated (Fig. 1). This observation supports our earlier hypothesis that after intranasal inoculation, a number of infectious foci are established in the pharynx and that this number is related to the dose of virus given (McVicar et al. 1971). A lower dose would establish fewer foci, and more time would be required before virus release exceeded virus clearance.

Increasing titres during the growth period indicated an increasing rate of virus release until an equilibrium was established as indicated by a levelling off of the curve. Recurrent peaks of many of the curves at about 6 hr. intervals suggest that the release of the virus is cyclic.

Three of the 10 non-immunized cattle (Fig. 2), two of the vaccinated cattle (Fig. 5, Steers 16 and 17), and one steer inoculated with heterologous virus (Fig. 7, Steer 27) had lower apparent rates of pharyngeal virus growth than the others. The vaccinated cattle did not become diseased after virus exposure, and the onset of clinical signs was somewhat delayed in the other four. The cause of this phenomenon is not understood, but cell-related factors influencing growth rates could be the efficiency of the adsorption and penetration of the virus, the time required for assembly and release of infectious virus, and the number of virus progeny produced by each cell. Other factors would be associated with the ability of the virus to reach and infect other cells, such as the clearance rate and the presence of specific of non-specific neutralizing activity in the extracellular fluids, interferon, and co-infection of cells with other agents.

The classification of the immune status of vaccinated cattle on the basis of serum MPI was proposed by Cunha *et al.* (1957). In their study, the immunity of cattle was challenged by the inoculation of 10^4 mouse LD50 of virus into the tongue epithelium, and they found that generalization rarely occurred in cattle with an MPI $\geqslant$ 2·0. Cattle with an MPI between 1·0 and 1·9 might or might not resist, and those with an MPI < 1·0 were not expected to be protected. Because cattle in the present experiment with MPI's between 1·0 and 3·8 developed clinical reactions, a retrospective study was made to determine whether these reactions were the rule after upper respiratory exposure. A group of 49 cattle immunized with either oil adjuvant or aluminium hydroxide gel vaccine and then exposed to the homologous virus strain was surveyed with the following results: clinical signs developed in 13 of 19 (69 %) cattle with a pre-exposure serum MPI > 2·0; six of 17 (35 %) cattle with an MPI 2·0–3·9; and one of 13 (8 %) with an MPI $\geqslant$ 4·0. These results suggest that exposure to virus by intranasal inoculation or contact with infected cattle can provide a more rigorous challenge of immunity than tongue inoculation.

The early growth of virus in immunized cattle has been shown to result in the release of viral progeny in quantities sufficient to depress circulating neutralizing activity. Serum antibody titres were shown to reach a low point about 4 days after virus exposure (Sutmoller & McVicar, 1972). Interestingly, titres of virus in the OP fluid of vaccinated and recovered cattle in the present study tended to reach a peak at 4 days after inoculation.

The extremely mild clinical syndrome exhibited by some of the vaccinated cattle after virus inoculation could easily have been missed under field conditions. Virus titres in OP fluid samples taken 2–4 days after inoculation from the four vaccinated steers with a low pre-exposure serum MPI were as high as those seen in the non-immunized cattle. Graves *et al.* (1971) have shown that non-immunized cattle transmit FMD virus with greatest efficiency, and Sellers & Parker (1969) have shown that virus titres in the surrounding air are highest at the time when clinical signs are beginning to appear. Therefore, the high virus titres seen in vaccinated cattle in the absence of obvious clinical signs suggest that partly immunized cattle, after exposure to virus, may become inapparent virus shedders and, therefore, dangerous sources of infection.

The depressed 2–4 day titres of virus in the OP fluid of vaccinated cattle with an intermediate or high pre-exposure serum MPI (Fig. 5, Steers 14 to 17) were probably related in some way to immunization. Treatment of these samples with trichlorotriflouroethane (TTE), which is capable of dissociating certain virus-antibody complexes (Brown & Cartwright, 1960), did not cause an increase in titre; this result suggests that either less virus was released into the OP fluid or that the virus released was rendered non-infectious by some means not reversible by TTE treatment.

Nine of 14 recovered cattle had neutralizing activity in the OP fluid at the time of re-inoculation with the homologous virus. Virus was never isolated from four of these cattle, even from OP fluid samples taken 20 min after re-inoculation. Sporadic isolations of small amounts of virus were made from the other five

31

steers. Virus was regularly isolated, on the other hand, from the five recovered cattle that had no detectable neutralizing activity in the OP fluid at the time of re-inoculation.

Treatment with TTE did not affect virus isolation from samples taken up to 30 hr. after inoculation from recovered cattle, but usually did increase the titres of samples taken at 2 to 7 days. These results suggest a secondary response of the local antibody system because the enhancing effect of TTE treatment is seen only with samples taken after 7 days from susceptible or vaccinated cattle. Neutralizing activity in the serum and the OP fluid of five of the recovered cattle was determined for 4 weeks after re-exposure as part of another study (McVicar & Sutmoller, 1974). A secondary response of circulating and local antibody was demonstrated in all five cattle, including one from which virus was never isolated. The inoculated virus stimulated antibody formation in this steer, and virus had probably multiplied even though it was never isolated from the OP fluid. Growth of homologous virus has also been shown in the mammary gland of recovered cattle after virus was infused through the teat canal (Burrows *et al.* 1971).

REFERENCES

Bachrach, H. L., Callis, J. J., Hess, W. R., Patty, R. E., DeBoer, C. J. & Hamblet, F. E. (1962). Large-scale production of bovine kidney cultures for plaque assay of foot-and-mouth disease virus and its ribonucleic acid. *American Journal of Veterinary Research* **23**, 608.

Brown, F. & Cartwright, B. (1960). Purification of the virus of foot-and-mouth disease by fluorocarbon treatment and its dissociation from neutralizing antibody. *Journal of Immunology* **85**, 309.

Burrows, R. (1966). Studies on the carrier state of cattle exposed to foot-and-mouth disease virus. *Journal of Hygiene* **64**, 81.

Burrows, R. (1968). Excretion of foot-and-mouth disease virus prior to the development of lesions. *Veterinary Record* **82**, 387.

Burrows, R., Mann, J. A., Grieg, A., Chapman, W. G. & Goodridge, D. (1971). The growth and persistence of foot-and-mouth disease virus in the bovine mammary gland. *Journal of Hygiene* **69**, 307.

Callis, J. J. & Cottral, G. E. (1968). Methods for containment of animal pathogens at the Plum Island Animal Disease Laboratory. In *Methods in Virology* ed. K. Maramorosch and H. Koprowski, vol. IV. 465 pp. New York: Academic Press.

Cunha, R. G., Baptista, J. A., Jr., Serrao, U. M. & Torturella, I. (1957). El uso de los ratones lactantes en la evaluacion de los anticurerpos contra el virus de la fiebre aftosa y su significacion immunologica. *Gaceta Veterinaria* **19**, 243.

Donaldson, A. I., Herniman, K. A. J., Parker, J. & Sellers, R. F. (1970). Further investigations on the air-borne excretion of foot-and-mouth disease virus. *Journal of Hygiene* **68**, 557.

Eskildsen, M. K. (1968). Experimental pulmonary foot-and-mouth disease infection of cattle. *Report of the meeting of the Research Group of the Standing Technical Committee, European Commission Control of Foot-and-mouth Disease, Food and Agriculture Organization of the United Nations, Lindholm, Kalehave, Denmark* 93,

FOGEDBY, E. G., MALMQUIST, W. A., OSTEEN, O. L. & JOHNSON, M. L. (1960). Air-borne transmission of foot-and-mouth disease virus. *Nordisk Veterinaermedicin* 12, 490.

GRAVES, J. H., MCVICAR, J. W., SUTMOLLER, P. & TRAUTMAN, R. (1971). Contact transmission of foot-and-mouth disease from infected to susceptible cattle. *Journal of Infectious Diseases* 123, 386.

HYSLOP, N. ST G. (1965a). Secretions of foot-and-mouth disease virus and antibody in the saliva of infected and immunized cattle. *Journal of Comparative Pathology* 75, 111.

HYSLOP, N. ST G. (1965b). Air-borne infection with the virus of foot-and-mouth disease. *Journal of Comparative Pathology* 75, 119.

MCVICAR, J. W., GRAVES, J. H. & SUTMOLLER, P. (1971). Growth of foot-and-mouth disease virus in the bovine pharynx. *United States Animal Health Association Proceedings* 74, 230.

MCVICAR, J. W. & SUTMOLLER, P. (1969). The epizootiological importance of foot-and-mouth disease carriers. II. The carrier status of cattle exposed to foot-and-mouth disease following vaccination with an oil adjuvant inactivated virus vaccine. *Archiv für die gesamte Virusforschung* 26, 217.

MCVICAR, J. W. & SUTMOLLER, P. (1974). Neutralizing activity in the serum and oesophageal-pharyngeal fluid of cattle after exposure to foot-and-mouth disease virus and subsequent re-exposure. *Archiv für die gesamte Virusforschung* 44, 173.

MCVICAR, J. W., SUTMOLLER, P. & ANDERSEN, A. A. (1974). Foot-and-mouth disease virus: plaque reduction neutralization test. *Archiv für die gesamte Virusforschung* 44, 168 .

MIRCHAMSY, H. & RAPP, F. (1968). A new overlay for plaquing animal viruses. *Proceedings of the Society for Experimental Biology and Medicine* 129, 13.

SELLERS, R. F. & PARKER, J. (1969). Air-borne excretion of foot-and-mouth disease virus. *Journal of Hygiene* 67, 671.

SUTMOLLER, P., COTTRAL, G. E. & MCVICAR, J. W. (1968). A review of the carrier state in foot-and-mouth disease. *United States Livestock Sanitary Association Proceedings* 71, 386.

SUTMOLLER, P. & MCVICAR, J. W. (1972). Foot-and-mouth disease: Changes in serum-neutralizing activity of immunized cattle shortly after virus exposure. *Infection and Immunity* 6, 718.

SUTMOLLER, P. & MCVICAR, J. W. (1973). Foot-and-mouth disease: Growth of the virus after conjunctival inoculation of cattle. *Archiv für die gesamte Virusforschung* 43, 284.

SUTMOLLER, P., MCVICAR, J. W. & COTTRAL, G. E. (1968). The epizootiological importance of foot-and-mouth disease carriers. 1. Experimentally produced foot-and-mouth disease carriers in susceptible and immune cattle. *Archiv für die gesamte Virusforschung* 23, 227.

VANBEKKUM, J. G., FRENKEL, H. S., FREDERIKS, H. H. J. & FRENKEL, S. (1959). Observations on the carrier state of cattle exposed to foot-and-mouth disease virus. *Tijdschrift voor Diergeneeskunde* 84, 1159.

VANBEKKUM, J. G., STRAVER, P. J., BOOL, P. H. & FRENKEL, S. (1966). Further information of the persistence of infective foot-and-mouth disease virus in cattle exposed to virulent virus strains. *Bulletin, Office International des Épizooties* 65, 1949.

WAGNER, G. G., CARD, J. L. & COWAN, K. M. (1970). Immunochemical studies of foot-and-mouth disease. VII. Characterization of foot-and-mouth disease virus concentrated by polyethylene glycol precipitation. *Archiv für die gesamte Virusforschung* 30, 343.