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Inhalation, persistence and dispersal of foot-and-mouth disease virus by man

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SUMMARY

Sampling of human subjects, who had been in contact with animals infected with foot-and-mouth disease (FMD) virus, showed that virus could be recovered from the nose, throat, saliva and from air expelled during coughing, sneezing, talking and breathing. The amounts of virus recovered paralleled those collected with a large-volume sampler and multistage impinger and these findings confirmed that the highest recovery of airborne virus was from infected pigs followed by cattle and sheep. More virus was found in the noses of those examining infected animals than in those operating the samplers, but there was variation between the subjects. In the majority there was a 1·8 log fall in titre by 3·5 hr., but virus persisted in the nose of one subject for 28 hr. Nose blowing or washing the nostrils did not remove virus completely, nor were cloth or industrial masks completely effective in preventing inhalation of virus. It was possible to transmit virus from infected subjects to others on one occasion. No clinical cases of FMD in man resulted from exposure, nor was there any rise in antibody. Use was made of these findings in determining sites of aerosol excretion in animals, and the results are discussed in relation to FMD in man and to the spread of respiratory viruses by the airborne route.

INTRODUCTION

In previous papers (Sellers & Parker, 1969; Donaldson, Herniman, Parker & Sellers, 1970) we gave the results of sampling air in boxes where cattle, sheep or pigs infected with foot-and-mouth disease were housed. During the sampling period one or more of us and our assistants had to remain in the box for up to 1 hr. to operate the large-volume sampler. At completion we sampled our noses, throats and saliva, and found FMD virus. These findings led to further investigations on the amount, persistence and dispersal of virus by man, and the results are recorded in this paper.

MATERIALS AND METHODS

The types of animals, methods of infection, air sampling and virus assay of aerosols from infected animals have already been described (Sellers & Parker, 1969; Donaldson *et al.* 1970).

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Viruses

The strains of FMD virus used were: O_1 BFS 1860, O_1 Swiss 1/66, O_2 Brescia, A_5 Eystrup (Tübingen), A_{22} Iraq 24/64, C Lebanon 3/69 and C Noville.

Collection of samples

Materials from the nose and throat were collected on dry bacterial swabs or cotton buds which took up 0·001 to 0·059 g. (mean 0·021 g.) of secretion and placed in 4 or 5 ml. of phosphate buffered saline (PBS) containing 0·5 % serum bovine albumin (PBS solution). Saliva (volume 0·1–0·45 ml., mean 0·24 ml.) was collected in 1 oz. bottles and diluted to 5 ml. with PBS solution. Plastic bags, into which people had sneezed, coughed, talked or breathed, were washed with 10 ml. PBS solution. Paper handkerchiefs used for nose blows contained 0·005–0·435 g. (mean 0·137 g.) of secretion and were placed in 1 oz. bottles containing 10 ml. PBS solution. On occasions nasal washings were obtained by introducing 2 ml. of PBS solution into each nostril. Samples were kept at 4° or −70° C. until assayed in calf thyroid cell cultures or unweaned mice.

Serum was collected at intervals from people exposed to infection and was tested for neutralizing antibody by the constant serum-varying virus method. Mixtures of virus and serum were held at 4° C. for 24 hr. before inoculation into unweaned mice.

RESULTS

Comparison of methods of sampling

Table 1 contains the results of several experiments, where the air in boxes holding infected animals was sampled with the large volume sampler, by nasal swabs and on one occasion with the multistage impinger. The titre of the virus recovered in the nasal swabs from people examining animals (examiners) was on all occasions higher than from people operating samplers (collectors) in the box, as might be expected from the close proximity to the animals together with the disturbance created during capture and restraint. The difference varied from 0·4

Table 1. *Comparison of virus recovery by various methods*

Species	Strain	Hours after infection	Virus recovery: Large vol. sampler	Virus recovery: Multistage impinger	Virus recovery: Nasal swabs: Collectors	Virus recovery: Nasal swabs: Examiners
Pig (8 pigs)	O_1 Swiss	70	6·3*	Stage 1, 3·95* Stage 2, 3·8 Stage 3, 3·55	3·4*	4·1*
	A_5	46	5·6	—	3·3	3·7
Sheep (8 sheep)	O_2	22	3·0	—	1·2	1·6
	C Noville	22	4·6	—	1·4	3·9
Cattle (2 cattle)	A_5	46	3·0	—	0·9	1·5
	C Noville	70	3·6	—	1·0	2·3

* Log ID 50 per collection.

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to 2·5 log. units; over a number of experiments the average difference was from 5- to 11-fold. The amounts of virus recovered from collectors paralleled those found with the large volume sampler; namely that most virus was in the boxes containing infected pigs followed by those containing cattle and sheep. The 2·3–3·2 log. units difference between the two methods was greater than the two log. units expected from the collecting rates: i.e. the large volume sampler operating at 1000 l./min. as compared to man breathing at 10 l./min. However (in Table 2) it is shown that the amount of virus in the nose did not increase after the first 5 min. of exposure probably owing to equilibrium between intake and clearance. There was variation between the amounts recovered from individual collectors and examiners of the order of 30- to 40-fold (Table 3).

Table 2. *Titres of virus recovered at intervals by various methods from eight pigs infected with* O_1 *Swiss* 1/66

Time of collection (min.)	Virus recovery: Large vol. sampler	Multistage impinger	Nasal swab: Collectors	Nasal swab: Examiners
5	—	—	3·6*	—
10	—	—	3·0	—
15	5·1*	—	3·3	—
30	5·7	—	—	—
45	5·5	Stage 1, 4·15* Stage 2, 3·0 Stage 3, 3·2	3·0	After 45 min. 4·2

* Log ID 50 per collection.

Table 3. *Variation in amount of virus recovered from the nose*

	Nasal swabs
Collectors: 2·1*, 2·5, 2·9, 2·9, 3·1, 3·3, 3·3, 3·7	Mean 2·98
Examiners: 2·9, 3·15, 3·65, 4·15, 4·4	Mean 3·65

* Log ID 50 per sample.

Dispersal of virus by various activities

As well as the taking of nasal swabs after examination of pigs, noses were blown, throats swabbed and saliva collected: plastic bags were used to collect the air from sneezes, coughs, talking and breathing. Some of the results are shown in Table 4. The amount recovered from nose blows was similar to that recovered in nasal swabs, whereas less virus was found in the throat and saliva. Virus was recovered on many occasions from coughs, sneezes, talking and breathing (Table 5).

Persistence of virus in nose

When subjects remained in the vicinity of infective animals, the concentration of virus found in the nose did not vary greatly (Table 2). In further experiments nasal swabs were taken at intervals after exposure to infected animals. Between

the first and second nasal swab the subjects had had a shower and made two changes of clothing. The rate of disappearance of virus varied from person to person. On average there was a fall in virus titre of 1·8 log. units in 3·5 hr. (Table 6). With one person virus was recovered 28 hr. after exposure, but no virus was recovered at 48 hr.

Table 4. *Amounts of virus recovered in nasal swabs, nose blows, throat swabs and saliva*

	Nasal swabs	Nose blows
Expt. 1	2·65*, 3·4, 3·65, 4·15 Mean 3·46	2·7, 3·2, 3·25, 4·15 Mean 3·33
Expt. 2	3·5, 3·8, 4·7, 4·7 Mean 4·17	3·4, 4·2, 4·4, 4·6 Mean 4·15

	Nasal swabs	Throat swabs	Saliva
Expt. 3	4·1, 4·1, 4·1	⩽ 0·9, ⩽ 0·9, 1·1	1·1, 2·3, 1·7
Expt. 4	2·7, 3·1, 3·1, 3·3	1·3, 1·3, 1·75, 2·5	—

* Log ID50 per sample.

Table 5. *Dispersal of virus by various activities*

		Mean
Nasal swabs	3·1*, 4·15, 4·2, 4·4	3·96
Coughing (3 times)	⩽ 1·2, 2·2, 1·8, 2·4	1·9
Nasal swabs	2·6, 3·65, 4·7	3·65
Sneezing (3 times)	⩽ 1·9, 3·2, 3·4	2·8
Nasal swabs	2·5, 2·7, 3·0, 3·1, 3·5	2·96
Talking (1 min.)	⩽ 1·2, ⩽ 1·2, 1·4, ⩽ 1·2, 3·6	⩽ 1·72
Nasal swabs	2·7, 2·7, 3·6, 3·7, 3·8	3·3
Breathing (1 min.)	⩽ 1·2, ⩽ 1·2, 2·6, ⩽ 1·2, 2·6	⩽ 1·76

* Log ID50 per sample.

Table 6. *Clearance of virus from nose*

		Time after exposure (hr.)			
	Subject	0	2·5–4·5	22–24	28
Expt. 1	1	2·1*	⩽ 0·9	⩽ 0·9	⩽ 0·9
	2	2·5	⩽ 0·9	⩽ 0·9	⩽ 0·9
	3*a*	2·9	1·1	⩽ 0·9	⩽ 0·9
	4*a*	2·9	1·5	⩽ 0·9	⩽ 0·9
	5*a*	3·1	1·1	⩽ 0·9	⩽ 0·9
	5*b*	3·1	1·5	⩽ 0·9	⩽ 0·9
	6	3·3	1·1	⩽ 0·9	⩽ 0·9
	7	3·3	1·2	⩽ 0·9	⩽ 0·9
	4*b*	3·5	1·3	⩽ 0·9	⩽ 0·9
	8	3·7	1·0	1·2	1·3

		0	1	2	3	5
Expt. 2	5*c*	4·1*	3·0	—	2·3	1·75†
	3*b*	4·1	2·3	1·7	⩽ 0·9	1·1†

* Log ID50 per nasal swab. † Log ID50 per nasal washing.

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Removal of virus from nose

Various methods of removal of virus from the nose were attempted. After one nasal swab, the titre fell by 0·35 to 0·75 log. units: after swabbing the nostrils with swabs soaked in 1/1000 citric acid or tap water a loss of 0·6–1·0 log. units was noted. A single nose blow resulted in a reduction of 0·5 log. units but even after 10 nose blows virus was still detectable (Table 7). Wearing a surgical cloth* or an industrial gauze and cotton wool† mask reduced the amount of virus inhaled by 0·9 or 0·8 log. units (Table 8) but paper* masks had no effect.

Table 7. *Effect of nose blowing on virus remaining in nose*

	Before nose blow	After 1 nose blow	After 10 nose blows
Expt. 1	3·0*, 3·15, 3·4, 3·65 Mean 3·3	2·65, 2·4, 2·9, 3·15 Mean 2·8	—
Expt. 2	2·5, 2·7, 2·9, 2·9, 3·2 Mean 2·8	—	1·3, 2·0, 1·8, 2·5, 1·9 Mean 1·9

* Log ID 50 per nasal swab.

Table 8. *Effect of masks in preventing inhalation of virus*

Type of mask			Mean
Cloth	With	2·6*, 3·1, 3·2	3·0
	Without	3·6, 4·2, 3·9	3·9
Industrial	With	1·8, 2·8, 2·8	2·5
	Without	2·8, 3·4, 3·6	3·3

* Log ID 50 per nasal swab.

Transfer of virus

Four attempts were made to transfer FMD virus. Three people examined infected pigs and then talked with colleagues in a box at the other end of the isolation unit for two minutes on two occasions. In three of the four attempts no virus was recovered before or after exposure, but on one occasion $10^{1·3}$ ID 50 was recovered from the nose of a recipient after the period of four minutes.

Antibody titres in persons exposed to FMD virus

Serum was taken from subjects before and 3 weeks after exposure. No significant titre or rise of antibody was detected, although one person showed neutralizing titres of 2·4 and 2·6 respectively before and after exposure to C Noville virus. No activity against FMD virus was found in nasal washings. Exposure to FMD virus did not prevent the development of respiratory illnesses in five out of eight subjects.

* Robinson and Son Ltd., Chesterfield.
† Martindale Electric Co. Ltd., London, N.W.10.

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DISCUSSION

It is not surprising that after exposure to infected air FMD virus was found in the nose. The amounts found can be correlated with those recovered by other means provided that allowance is made for the rapid uptake by the nose and subsequent clearance. The results obtained from nasal swabs confirm those previously found with the large volume sampler, namely that the greatest amount of airborne virus is excreted by pigs, followed by cattle and sheep, and that sheep excrete virus although no lesions are apparent.

The higher amount of virus collected during examination of the animals may be attributed to closer proximity and the disturbance set up in catching and holding the animals. Use was made of the greater mobility of the nose compared with that of the large volume sampler in assessing the sites of origin of the airborne virus. In preliminary experiments we found that even when extensive vesicles were present on the tongue of steers the day after inoculation, no virus was recovered from the nose of an examiner unless generalization of the disease to the lips or other sites of the animal had occurred. In another experiment we exposed examiners to the heads of the pigs and to the remaining parts of the pigs; the parts not sampled were held in a plastic bag. Although lesions were present on the feet as well as on the tongue and snout, more virus was recovered from the noses of those examining the head. These preliminary results suggest that the source of airborne virus might be some part of the upper respiratory tract. No virus has been recovered from people examining infected mice or guinea-pigs. Gibbs (1931) found that guinea-pigs did not readily transmit virus to others in the same cage, but that hedgehogs did. Edwards (1934) recovered virus from the breath of infected hedgehogs and demonstrated multiplication of FMD virus in the nasal mucous membranes. He failed to recover virus from the breath of infected guinea-pigs. It may be that examination of infected hedgehogs might lead to recovery of virus from the examiner's nose. At the laboratory bench, virus ($10^{2\cdot9}$ ID 50) was recovered from the nose of one of us immediately after the collection of supernatant fluids from BHK 21 cells in Roux bottles infected with C Noville virus.

Inhalation of such large amounts of virus over a period has not resulted in any clinical signs of foot-and-mouth disease in Institute workers who have handled animals infected with foot-and-mouth disease. The results of our antibody tests revealed that one of 10 sera examined showed some antibody to type C, and there was no conversion to positive as a result of exposure. This is not surprising as FMD in man is rare. In reported cases of FMD in man (Vetterlein, 1954; Heinig & Neumerkel, 1964; Pilz & Garbe, 1965; Armstrong, Davie & Hedger, 1967; Eissner, Böhm & Jülich, 1967; Suhr Rasmussen, 1968) the source of infection was attributed to drinking infected milk, accidental self-inoculation or to handling infected animals while the skin was damaged by cuts, manicure or by dermatitis. It is likely that in some instances the patients would have inhaled virus in reasonable amounts and infection could also be ascribed to this route. Of the 37 cases reported 23 were due to type O, 13 to type C and 1 to type A virus. In a serological survey of workers exposed to FMD virus, Suhr Rasmussen (1968) and Wisniewski &

Jankowska (1968) found that low titre antibodies to type O were the most prevalent followed by type C. In this connexion it is interesting to note that the highest yields of airborne virus were recovered from animals infected with O_1 or C Noville strains of virus (Donaldson, *et al.* 1970).

The inhalation, retention, clearance and dispersal of particles in the nasal region has been extensively investigated by the use of physical and chemical substances as well as bacteria and viruses (Buckland & Tyrrell, 1964; Buckland, Bynoe & Tyrrell, 1965; Druett, 1967). In many of these investigations subjects were exposed to artificially generated aerosols or materials such as spores or viruses were placed in the nostrils. The materials varied in their adhesive properties; some, such as pollen or fungal spores, showed greater penetration, whereas others adhered to the nasal hairs or attached themselves to cells. With rhino- and Coxsackie-viruses, infection of cells together with virus multiplication and production of nasal secretion took place. Our results were obtained by exposure of subjects to a naturally generated aerosol, measurements of which were made to determine virus recovery and particle size. In addition FMD virus is similar in certain properties to rhinoviruses, e.g. pH lability and size. Unlike rhinoviruses, FMD virus did not multiply in the nose but may have attached to nasal hairs and cells. Our results may therefore represent some of the aspects of initiation of infection by respiratory viruses before multiplication has occurred and fill a gap between the results of Buckland & Tyrrell (1964) with bacterial spores and bacteriophage and those of Buckland *et al.* (1965) and Gerone *et al.* (1966) with Coxsackie virus A 21.

On exposure to infected animals the nose rapidly took up FMD virus, but the virus concentration did not increase after 5 min. while the subject remained in the particular environment. When the subject moved to a higher concentration of virus, e.g. from cattle boxes to pig boxes, the amount of virus in the nose increased until an equilibrium was reached. When he moved out of the infected environment, the amount in the nose decreased at the rate of 1·1 to 1·8 log. units in an hour. This was slower than the fall in titre recorded for *Bacillus mycoides* spores, namely 1·4 and 2·6 log. units in 40 min. (Buckland & Tyrrell, 1964). It may be that FMD virus attached better to cells than the spores, and this suggests that a longer period is available for respiratory viruses to attach. In addition with FMD virus clearance may not be complete, since even after 10 nose blows virus was recovered in nasal swabs. Activities such as nose blowing or snorting may represent another chance for virus to attach to cells. The amount of FMD virus recovered from nose blows was the same as from nasal swabs, whereas volunteers infected with Coxsackie virus A 21 shed more virus in the nose blows than in the nasal washings. This difference may be due to the nose blow bringing forward secretion from an actively multiplying site of A 21 virus. In our experiments on an average seven times more nasal secretion was found in a nose blow than in a nasal swab. Since the virus titres were the same, this probably means that a certain proportion of virus is attached to the cells and cannot be removed by the nose blows. The amounts of virus recovered in saliva and throat swabs were less than the amounts recovered in nasal swabs as demonstrated by Buckland & Tyrrell (1964). Sneezes were considered to be good dispersers of virus and in our experiments gave good recovery,

although it must be pointed out that we measured only the amounts attached to the wall of the plastic bag and not the enclosed air. We also recovered virus from breathing and talking on two occasions, although these activities are not considered good dispersers of bacteria or viruses. Tyrrell (1967) has pointed out that in respiratory infections virus may be spread by individuals who excrete particularly high concentrations of virus. In our experiments, despite comparable exposure, there was variation between subjects in the amount of virus in nasal and other secretions and in the rate of clearance. It may be that this variation in anatomical and physiological factors may influence the chance of infection and spread of respiratory viruses.

No experiments have been done to see whether FMD can be transferred from one animal to another through exhalation of virus by man. However, when people who had been examining infected animals talked to colleagues for 4 min., virus was subsequently recovered from the nose of one of the colleagues, although the virus could have come from the clothing of the examiners as well as from the exhaled air. This is a further indication of how rapidly a respiratory virus may be transmitted. Industrial, surgical or paper masks were not effective in protecting the nose from inhalation of virus. It could be argued that only the smaller particles were penetrating; on the other hand virus was still recovered from the nasal passages, where only particles greater than 6 μ are retained (May, 1966) so it would appear that a proportion of the larger particles were entering the nose. To assume therefore that such masks afford protection against inhaling or exhaling infective virus is false; the only effective method of protection would be provided by respirators capable of trapping large and small particles. To avoid transferring FMD virus it would be advisable to allow natural clearance of virus from the nose. Virus swallowed would be rapidly inactivated by the low pH in the stomach.

Our results emphasize that the respiratory tract of man or animal is a most effective device for sampling aerosols, and details of the amounts recovered may be used to determine whether multiplication of virus has occurred either in man exposed to respiratory viruses or in animals exposed to FMD.

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