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Airborne excretion of foot-and-mouth disease virus

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Foot-and-mouth disease (FMD) virus may spread in a number of ways: by direct contact between infected and susceptible animals, by animal products such as meat and milk, by mechanical transfer on people, non-susceptible animals, birds, vehicles and fomites, and by the airborne route. The initial pattern of outbreaks at the beginning of the epidemic in the West Midlands of England in 1967 suggested that spread was airborne. The meteorological evidence for this and for past epidemics in Great Britain has been investigated by L. P. Smith, P. B. Wright and M. Hugh-Jones (personal communications, 1968–9) and by Hurst (1968). Henderson (1969) has also studied the spread of disease in the Worcestershire area and attributed much of it to wind carriage. Methods and results of aerosol sampling of virus and infected cattle were reported by Thorne & Burrows (1960) and by Hyslop (1965). In this paper the results are given of the measurement of virus in aerosols produced by cattle, sheep and pigs infected with FMD virus.

MATERIALS AND METHODS

Animals

The cattle were 18-month-old Devon steers about 400 kg. in weight. Crossbred sheep were about 30 kg. in weight and Large White pigs 30–40 kg. They were housed (two cattle, eight sheep or eight pigs per box) in looseboxes 3·65 m. × 3·35 m. × 3·05 m.

Virus

Four strains of FMD virus were used: 0_1 Lombardy, 0_1 Swiss 1/66, 0_1 BFS 1860 and 0_2 Brescia. They had been passaged in cattle or pigs or given one passage in IB-RS-2 tissue cultures (de Castro, 1964).

Infection of animals

Cattle were inoculated intradermally at four sites on the tongue, sheep on the coronary band of one foot and pigs on the bulbs of the heel of one foot with 10^4 to 10^5 ID 50 of virus. The animals were observed daily and the extent of lesions noted. In one experiment with the multistage impinger pigs were exposed to infection by being placed in the same box as inoculated pigs and subsequently removed.

Air sampling

The air in the box was sampled once or twice daily by drawing through a large-volume sampler (Litton Model M (Modified), Litton Systems Inc., Minneapolis, Minnesota, U.S.A.). The sampler was run for 1 hr. at 1000 l./min. with a collecting

fluid of phosphate buffered saline (PBS) containing 5 % inactivated ox serum which had been screened for the absence of substances inhibitory to FMD virus. Between runs the sampler was disinfected and cleaned by pumping through 0·2 % citric acid in distilled water, followed by detergent, distilled water and PBS. At the time of collection, the air inlet and outlet in the box were blocked. Air in the central area of the isolation unit was also sampled.

In experiments to determine particle size, a multistage liquid impinger (May, 1966) was run for 45 min. at 55 l./min. The sampling fluid was PBS and the apparatus was sterilized by autoclaving between samplings.

Temperature and relative humidity were recorded during sampling.

Virus assay

Samples were assayed by inoculation of unweaned mice (Skinner, 1951) and calf thyroid tissue culture tubes (Snowdon, 1966). Specificity of reaction was checked by complement fixation and further passage.

RESULTS

Conditions of operation of large-volume air sampler

To test whether loss of virus titre occurred during sampling, virus at varying concentrations was added to the sampling fluid and circulated in the large-volume sampler for an hour at a relative humidity of 55 % and a temperature of 21° C. About 25 % of the volume of fluid was lost through evaporation but no fall in virus titre was noted.

Four ml. of collecting fluid was taken every 15 min. up to 60 min. during sampling in a loosebox. With a high concentration of virus in the box maximum titre was found after 45 min., with a low concentration at 60 min.

Air in infected looseboxes was sampled with or without the electrostatic precipitator. No significant difference was found in the amount of virus collected.

Sampling of infected animals

The results of sampling the air of looseboxes containing cattle, sheep or pigs infected with strains of O_1 and O_2 virus are shown in Tables 1–3. The maximum amount of virus recovered per animal per sampling period was the same for cattle and sheep but was about 30-fold higher for pigs. Airborne excretion from pigs lasted 5 days and totalled about 10^6 ID 50 of virus per animal, whereas from sheep and cattle the total virus excretion per animal was about 3×10^4 ID 50 over 4 days. The time of maximum recovery from sheep took place before lesions were visible; in the majority of sheep, lesions were not visible or were difficult to find. Maximum recovery from cattle and pigs was found immediately after generalization from the site of inoculation had occurred but before vesicles had ruptured.

The temperature in the looseboxes varied from 8·5 to 18·5° C. and the relative humidity from 72 to 100 %.

Air was also sampled in the central area of the isolation unit outside the loosebox. When pigs were infected, titres of $10^{4·7}$ ID 50 per sample were found over a period of 2 days.

Table 1. *Extent of lesions and recovery of virus in the large-volume sampler from infected cattle*

Hours post-infection	Extent of lesions	No. of collections	Virus recovery: Range	Virus recovery: Mean	Virus recovery: Mean per animal
17	Unruptured vesicles on tongue	4	2·8–2·9*	2·85	2·55
22		4	2·8–3·2	3·0	2·7
41	Unruptured vesicles on tongue.	4	3·1–4·0	3·5	3·2
46	Vesicles developing on lips and feet	4	2·7–3·0	2·9	2·6
65	Ruptured vesicles on tongue and mouth, vesicles on feet	4	2·7–3·0	2·8	2·5
89	Ruptured vesicles on feet	4	< 1·9–2·8	< 2·3	—
113	Tongue healing. Ruptured vesicles on feet	4	< 1·9	< 1·9	—

* Total virus (log ID 50) recovered over 60 min. at 1000 l./min.

Table 2. *Extent of lesions and recovery of virus in the large-volume sampler from infected sheep*

Hours post-infection	Extent of lesions	No. of collections	Virus recovery: Range	Virus recovery: Mean	Virus recovery: Mean per animal
17	Pain in feet	3	3·65–4·5*	4·1	3·2
41	Swelling and discharge in inoculated foot	3	2·8–3·6	3·3	2·4
65	Lesions in uninoculated feet and in mouth (30 % of animals)	3	2·7–2·8	2·75	1·85
89	Feet healing, lameness	2	< 1·9–2·4	< 2·15	—
113	No change	2	< 1·9	< 1·9	—

* Total virus (log ID 50) recovered over 60 min. at 1000 l./min.

Table 3. *Extent of lesions and recovery of virus in the large-volume sampler from infected pigs*

Hours post-infection	Extent of lesions	No. of collections	Virus recovery: Range	Virus recovery: Mean	Virus recovery: Mean per animal
17	Vesicles in 25 % of sites inoculated	4	< 1·9	< 1·9	—
41	Vesicles at sites inoculated, vesicles on other feet	4	5·2–5·9*	5·6	4·7
65	Primary vesicles ruptured. Vesicles on feet, tongue, snout, mouth	4	4·8–5·5	5·2	4·3
89	Ruptured vesicles	4	3·6–4·2	3·9	3·0
113	No change	4	3·0–4·4	3·9	3·0
137	No change	4	2·7–2·9	2·8	1·9

* Total virus (log ID 50) recovered over 60 min. at 1000 l./min.

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Sampling with a multistage liquid impinger

The air in looseboxes containing infected pigs was sampled with a multistage liquid impinger and the results of one experiment are shown in Table 4. By analysis of variance the results are significant at the 5% level. On average, 65% of virus was recovered in the first stage, 24% in the second and 11% in the third; in a second experiment the percentages were 71, 19 and 10. The amount of virus recovered by the multistage impinger was of the same order as that recovered in the large-volume sampler, when allowance was made for rate and time of sampling.

Table 4. *Recovery of virus in stages of multistage liquid impinger from infected pigs*

	Hours post-infection							
	17	22	41	46	65	70	89	94
Stage 1	< 1·2	2·1*	3·95	3·1	3·3	3·2	3·3	2·8
Stage 2	< 1·2	< 1·2	3·3	3·0	2·8	3·1	2·6	2·2
Stage 3	< 1·2	< 1·2	3·35	2·2	2·6	2·4	2·55	1·85

* Total virus (log ID 50) recovered over 45 min. at 55 l./min.

DISCUSSION

The amount of virus recovered from the air of boxes containing infected cattle was similar to that found by Hyslop (1965), if allowance is made for the different rate of sampling. However, with the strains of 0_1 and 0_2 used, virus was not found after the 4th day whereas Hyslop (1965) described virus recovery up to the 14th day after infection with a strain of SAT 1. Kiryukhin & Pasechnikov (1966) recovered 6·3 to 630 ID 50 per litre of exhaled air from calves infected with type 0 virus. This is from 40- to 4000-fold higher than the maximum we obtained, but these authors collected from a mask attached to the animal. In the experiments described above, pigs excreted the greatest amount of virus over the longest period. With both pigs and cattle infected by injection maximum excretion was found when lesions at secondary sites were just visible. In pigs infected by contact maximum excretion was found at the same time as lesions were observed. With sheep maximum excretion occurred before even experienced observers had seen clear signs of disease and in the majority of sheep lesions did not develop, although virus was recovered in pharyngeal samples 7 and 14 days after infection.

The source of virus is uncertain. It is unlikely to be solely from rupture of lesions on the tongue and feet, since at the time of maximum collection lesions were not ruptured in cattle and pigs and had not yet been observed in sheep. Nor is it likely to be from excessive salivation, since this sign was not observed in the cattle until the 3rd day after infection. Virus was found in the pharynx (Burrows, 1968*a*; Sellers, Burrows, Mann & Dawe, 1968) and nasal mucous membrane (Korn, 1957) during the incubation period and it is probable that the virus, recovered as aerosol, came from these sites in the upper respiratory tract. As far as the lower part of the respiratory tract is concerned, Eskildsen (1969) described recovery of virus

from consolidated areas of the lung after infection by the intratracheal route and development of lesions. Other possible sources of virus are the skin of the animals and the dust and faeces in the box. Maximum titres of pig and sheep faeces ($10^{2.9}$ and $10^{2.7}$ ID 50 per g.) were found on the 2nd or later days after infection, and in cattle, titres of $10^{4.9}$ ID 50 per g. or greater were found from the 2nd to 5th day of infection. From these findings one would expect that if faeces were the source of airborne virus, cattle would excrete the greatest amount, but this was not the case. However, spreading of slurry of cattle faeces could set up an aerosol.

Unpublished experiments (G. J. Harper, J. N. Wilson & R. F. Sellers, 1968) showed that FMD virus survival in an aerosol depended on a high relative humidity, loss of infectivity occurring rapidly at a relative humidity of less than 70%. Provided this condition is satisfied and provided that inactivation from other causes does not take place, the concentration and dosage of virus downwind from a source can be calculated from Pasquill's (1961) formula. At a wind speed of 5 m./sec., a lateral spread of 10° and a vertical spread from 10 to 100 m. depending on distance, 100 pigs excreting $10^{2.9}$ ID 50 per pig per min. could give rise to a concentration of 5 ID 50 per 10^3 l. of air at 100 m., 5 per 10^4 l. at 1 km., 1 per 10^5 l. at 10 km., 1 per 10^6 l. at 50 km. and 5 per 10^7 l. at 100 km. One hundred cows or sheep would give rise to about thirty times less. If the upper limit of the range is taken, these concentrations could be doubled. It is not known how efficient the large-volume sampler is. With a larger model Gerone *et al.* (1966) state that recovery of generated aerosols of Coxsackie A 21 virus varied from 1 to 20%. If the same efficiency applies to the smaller sampler, the concentrations can be raised a further 5- to 100-fold.

The results with the multistage liquid impinger indicated that 65–71% of virus recovery was correlated with particles greater than 6 μ, 19–24% between 3 and 6 μ, and 10–11% less than 3 μ (May, 1966). Provided that these sizes are not changed during transport in air, on analogy with man these particles might be expected to infect the upper respiratory tract, bronchi and alveoli respectively. The minimum infecting dose is not known; Eskildsen (1969) reported that 10–100 mouse ID 50 were sufficient to set up infection when inoculated by the intratracheal route and Sutmöller, McVicar & Cottral (1968) infected six out of six animals by injection of tonsillar sinuses and two out of six by intranasal inoculation with 10^2 p.f.u.

The volume of tidal air breathed varies among animals sampling the aerosol. At rest a 400 kg. Holstein cow would sample 85 l./min., a 25 kg. pig 9·27 l. (Brody, 1945) and a sheep about 5 l. (Amoroso, Bell & Rosenberg, 1951). Over a period of 50 hr. 100 cattle would sample $2{\cdot}5 \times 10^7$ l., while 100 sheep or pigs would sample $1{\cdot}5$ to $2{\cdot}8 \times 10^6$ l. This would be sufficient to sample the concentration of virus specified at 100 km. over this period. Within species the tidal air volume in adults is greater than in young animals. One would therefore expect that, given the same concentration of virus in the air, the larger animal would be infected first. In an experiment (Burrows, 1968*a*) where cattle, sheep and pigs were placed in the same unit as infected animals, 50% of cattle had virus in the pharynx at 72 hr., 50% of sheep at 129 hr. and 50% of pigs at 130 hr. If in the field under conditions of

airborne spread animals are exposed to the same concentration of virus, it would be expected that larger herds or flocks would be infected first, that larger animals such as cattle would be infected before pigs or sheep, and adults before calves, piglets or lambs. However, after infection, lesions are observed earlier in cattle than in sheep or pigs (Tables 1–3, and Burrows, 1968*a*) and lesions in sheep may be difficult to detect (Results, and Burrows, 1968*b*); thus the picture would not be clear unless pharyngeal samples were taken for examination for virus. But if in the field lesions are observed in pigs and sheep at the same time as cattle, it is probable that pigs or sheep were infected first and the disease may then have spread to the cattle. If only cattle are affected and pigs or sheep are also on the same farm, it does not necessarily follow that the cattle were infected first, since pharyngeal sampling might establish that virus was also present in the pigs or sheep. Indeed, differential rate of infection together with the varying rate of lesion development may explain certain anomalies in the field, such as cattle showing lesions but not calves, and sheep but not lambs.

Cattle are generally regarded as the most important animals in the dissemination of foot-and-mouth disease because of the nature and extent of lesions and their high virus content and because of the high virus content in milk and faeces. However, in airborne transmission of virus the role of pigs and sheep is emphasized. Sheep act as maintenance hosts, pigs as amplifiers and cattle as indicators.

SUMMARY

A large-volume sampler was used to recover virus excreted as aerosol by cattle, sheep and pigs infected with foot-and-mouth disease. Pigs were found to excrete virus to a maximum of $10^{4.7}$ ID 50 per animal per hour and sheep and cattle to a maximum of $10^{3.2}$ ID 50. Excretion from pigs totalled 10^6 ID 50 per animal over 5 days and from cattle and sheep 3×10^4 ID 50 per animal over 4 days. Maximum recovery occurred 41 hr. after infection in pigs and cattle when lesions had generalized and 17 hr. after infection in sheep before lesions had been observed. Sampling in a multistage liquid impinger showed that 65–71 % of virus was excreted as an aerosol of size $> 6\ \mu$, 19–24 % 3–6 μ and 10–11 % less than 3 μ. The site of production of virus excreted as aerosol is suggested to be the upper respiratory tract. Under conditions of relative humidity greater than 70 % and at low temperatures, survival of virus to a distance of 100 km. is likely to occur and because of the minute respiratory volume the aerosol would be sampled more efficiently by cattle than pigs or sheep and by large animals than by small. These findings are discussed in relation to spread of virus in the field.

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