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The effects of spraying on the amounts of airborne foot-and-mouth disease virus present in loose-boxes

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SUMMARY

The air of loose-boxes which had previously held pigs infected with foot-and-mouth disease was sampled for virus after various procedures. Removal of infected pigs led to a 12- to 16-fold reduction in the amount of virus after 5 min. and a 400-fold reduction after 60 min. After heavy spraying (1·2 mm. of water in 5 min.) the amount of virus was reduced 500-fold compared to 30-fold after light spraying (0·20 mm. of water in 5 min.). The partition of infectivity associated with particle size was measured. The partition found after light spraying was similar to that found 5 min. after the pigs had been removed. Heavy spraying brought about a reduction in the infectivity associated with the large particles (> 6 μm.) but had no effect on particles less than 3 μm. A similar partition was found 60 min. after the pigs had been removed. The findings are discussed in relation to the spread of foot-and-mouth disease by the airborne route.

INTRODUCTION

When pigs infected with foot-and-mouth disease were held in a loose-box, large amounts of virus were recovered from the air, the highest percentage of infectivity being associated with particles greater than 6 μm. in diameter (Sellers & Parker, 1969). The present experiments were carried out in order to measure the concentration of virus and the association of infectivity with particle size after the air in the loose-box had been sprayed with water.

MATERIALS AND METHODS

Animals

Large White pigs, weight 30–40 kg., were housed in loose-boxes.

Viruses

Three strains of foot-and-mouth disease (FMD) virus were used: O_1 BFS 1860, A Pando and C Noville. They were stored at −70° C. as suspensions of infected cattle-tongue epithelium.

Virus assay

Virus was assayed by inoculating serial dilutions into calf thyroid tissue culture tubes.

Plan of experiments

After the infected pigs had been removed, measurements were made of the airborne virus remaining in the loose-box under the following conditions:

(i) When the box had been empty for 5 min.

(ii) When the box had been empty for 60 min.

(iii) When the air in the box had been sprayed with a heavy spray for 5 min.

(iv) When the air in the box had been sprayed with a light spray for 5 min.

These amounts were compared with the amount of airborne virus collected when the box contained infected pigs.

Experimental procedure

The pigs were inoculated on both forefeet with virus diluted 10^{-1} in phosphate buffered saline. The animals were observed daily. At 48 and 72 hr. after inoculation, when generalization of lesions had occurred, spraying and air sampling were carried out in the following sequence:

(1) The air inlets and outlets of the loose-box which housed the pigs were blocked and the walls were drenched with water to provide a relative humidity of greater than 95%.

(2) The air in the loose-box was sampled for 25 min. with the pigs present (see 'Loose-box containing infected pigs' in tables).

(3) The pigs were removed.

(4) The box was left for 5 or 60 min.

(5) The air in the box was sampled for 25 min. ('Empty loose-box 5 min.' and 'Empty loose-box 60 min.' in tables).

(6) The pigs were taken back into the box and the box was kept closed for 30 min. to recharge the air with virus.

(7) The air in the loose-box was sampled for 25 min. with the pigs present ('Loose-box containing infected pigs' in tables).

(8) The pigs were removed.

(9) The air in the box was sprayed with a heavy or light spray for 5 min.

(10) The air in the box was sampled for 25 min. ('Empty loose-box after heavy spraying' and 'Empty loose-box after light spraying' in tables).

On alternate occasions steps 9 and 10 were carried out after step 3, and steps 4 and 5 after step 8. In preliminary experiments and previously (Sellers, Herniman & Donaldson, 1971) it was found that the amounts of virus collected when the pigs were present in the box during steps 2 and 7 did not differ by more than 0·3 log units. This means that the period of 30 min. when the pigs were returned to the box (step 6) was sufficient to raise the amount of virus to the level at the beginning (step 2). Subsequently, sampling during step 2 was omitted; instead the pigs remained in the box for 1 hr. before being removed.

Air sampling

The air in the loose-boxes was sampled with a large-volume sampler (Litton Model M, Litton Systems Inc., Minneapolis, U.S.A.) and with a multistage impinger (May, 1966).

Table 1. *Percentage distribution of droplet size during spraying*

	Size of droplet (mm.)					
	< 0·5	0·5 to < 1·0	1·0 to < 1·5	1·5 to < 2·0	2·0 to < 3·0	⩾ 3·0
Heavy spray						
In air	53	39	6	1·4	0·4	0·2
On ground	26	55	13	4·0	1·0	1·0
Light spray						
In air	63	34·5	2·3	0·2	0	0
On ground	36	57	6·0	1·0	0	0

Spraying

The heavy spray was produced by forcing tap-water under pressure (2·8 kg./cm.^{2}) through a double-headed spray with nozzles of 1·6 mm diameter (Eclipse Spray Co. Ltd., Warley, Smethwick).

A Killaspray pressure-sprayer (ASL Super 828, ASL, Birmingham) was used to provide the light spray. Two litres of tap water were put in the container, which was pressurized with 80 strokes of the pump.

During spraying the sprays were held aloft with the jets directed towards the top of the loose-box and moved to and fro so that spray fell everywhere. Falling spray was collected through a filter funnel into a conical flask. The depth of water collected was calculated by dividing the volume by the area of the filter-funnel aperture.

Measurement of the size and distribution of the spray droplets was done by collecting the spray on Whatman No. 1 filter papers previously treated with potassium permanganate and correlating the size of splashes with the drop diameter derived from the weight of water required to produce them (Anderson, 1948). To give the distribution in the air, the number of drops of a particular size was divided by the terminal velocity (Best, 1950).

RESULTS

Amount of water sprayed and size of droplet

After 5 min. of spraying with the heavy spray, water to a depth of between 0·80 and 1·75 mm. (mean 1·21 mm.) was collected; after light spraying, 0·16–0·29 mm. (mean 0·20 mm.) was collected.

The percentage of droplets within each size range is shown in Table 1. The majority of droplets in the air were < 0·5 mm. in diameter, with rather more in the light spray than in the heavy spray.

Amount of virus collected

In Table 2 it can be seen that, as has previously been found (Sellers *et al.* 1971) removal of infected pigs from a loose-box reduced the amount of airborne virus by 12-fold to 16-fold (1·1–1·2 log units) after 5 min. and by 400-fold (2·6 log units) after 60 min. After heavy spraying, the amount was reduced 500-fold (2·7 log units)

Table 2. *Amounts of virus recovered in the large sampler from loose-boxes after spraying*

Loose-box containing infected pigs			Empty loose-box after 5 min.			Empty loose-box after heavy spraying		
4·4*	4·7	5·3	4·2	3·8	4·4	2·4	1·5	3·4
5·3	6·1	6·7	4·3	4·1	5·3	2·1	3·3	3·8
Mean 5·4			Mean 4·3			Mean 2·7		
Loose-box containing infected pigs			**Empty loose-box after 5 min.**			**Empty loose-box after light spraying**		
5·3	5·5	7·1	4·3	4·7	5·3	3·9	4·4	5·3
Mean 6·0			Mean 4·8			Mean 4·5		
Loose-box containing infected pigs			**Empty loose-box after 60 min.**					
4·7	6·1	6·7	2·7	3·1	3·9			
Mean 5·8			Mean 3·2					

* Log ID 50 per collection.

Table 3. *Amounts of virus recovered in the different stages of a multi-stage impinger from loose-boxes after spraying*

	Loose-box containing infected pigs	Empty loose-box after 5 min.	Empty loose-box after heavy spraying
Stage 1, > 6 μm.	3·2* 4·5 4·5 4·8 Mean 4·2	1·2 2·0 3·1 3·2 Mean 2·4	⩽ 1·0 1·2 1·2 1·5 Mean ⩽ 1·2
Stage 2, 3–6 μm.	3·2 3·5 4·2 4·2 Mean 3·8	1·5 2·2 1·8 3·4 Mean 2·2	⩽ 1·0 1·4 2·2 1·1 Mean ⩽ 1·4
Stage 3 < 3 μm.	3·9 2·6 3·2 4·6 Mean 3·6	1·5 2·6 3·2 3·2 Mean 2·4	1·5 2·4 2·6 2·2 Mean 2·2
	Loose-box containing infected pigs	**Empty loose-box after 5 min.**	**Empty loose-box after light spraying**
Stage 1, > 6 μm.	3·4 3·9 4·8 Mean 4·0	2·2 3·0 3·4 Mean 2·9	2·0 2·2 2·2 Mean 2·1
Stage 2, 3–6 μm.	2·6 3·9 4·4 Mean 3·6	1·9 2·8 3·0 Mean 2·6	2·0 2·2 2·2 Mean 2·1
Stage 3, < 3 μm.	2·6 3·5 4·0 Mean 3·4	1·9 2·8 2·8 Mean 2·5	2·2 2·0 2·2 Mean 2·1
	Loose-box containing infected pigs	**Empty loose-box after 60 min.**	
Stage 1, > 6 μm.	3·7 4·1 4·5 Mean 4·1	⩽ 1·0 1·5 1·9 Mean ⩽ 1·5	
Stage 2, 3–6 μm.	3·5 4·1 4·1 Mean 3·9	1·9 2·1 2·5 Mean 2·2	
Stage 3, < 3 μm.	2·8 3·0 3·2 Mean 3·0	2·1 2·3 3·3 Mean 2·6	

* Log ID 50 per collection.

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compared to 30-fold (1·5 log units) after light spraying. The reduction after heavy spraying was of the same order as the reduction after leaving the box empty for 60 min.

The results of experiments to determine the partition of infectivity between the different stages of a multi-stage impinger are given in Table 3. The partition, when infected pigs were present (63 %, 27 %, 10 % – mean of ten experiments), was similar to that previously found (Sellers & Parker, 1969). When the box had been empty for 5 min. the partition (mean of seven experiments with empty loose-boxes) was 43 %, 27 %, 30 %. After light spraying the percentages were 33·3 % for each stage, and after heavy spraying 9 %, 14 % and 77 % respectively. When the box had been empty for 60 min. the percentages were 5 %, 27 %, and 68 %.

DISCUSSION

Spraying a loose-box with water after removal of infected pigs reduced the concentration of FMD virus in the air, the heavy spray causing a greater fall in titre than the light spray. There was a selective action; the heavy spray brought about the greatest reduction in titre of the infectivity associated with particles $> 6\ \mu$m. but the infectivity associated with the small particles remained almost the same. In this respect the spraying intensified the loss of the larger particles from the air by sedimentation, which was taking place when the pigs were removed from the loose-box.

Spraying may simulate the action of rain. If so, rain would have no effect on the smaller particles and the larger particles would be washed out only by heavy rain. Such an effect would be predicted from previous work on wash-out of particles by rain, where the efficiency of capture by raindrops falls off very sharply for particles of 5 μm. or less (Chamberlain, 1967).

The site of initial infection appears in cattle to be the upper respiratory tract, especially in the pharyngeal area (Sutmöller, McVicar & Cottral, 1968; McVicar, Graves & Sutmöller, 1970; Burrows *et al.* 1971). The larger particles would be expected to be trapped here, the smaller particles being taken into the bronchi and alveoli (May, 1966). The fate of the large particles is therefore important in determining the outcome of airborne spread of foot-and-mouth disease.

The minimum infective dose for cattle by inhalation is 10^1 ID 50 and by ingestion 10^6 ID 50 (Sellers, 1971). Animals downwind could be infected in two ways; by breathing in the large particles present in the air or by breathing in droplets formed by the splash of heavy rain on impaction. Infection by ingestion of contaminated herbage would be less likely, even if under some circumstances there is a 350:1 ratio in favour of the dose available for ingestion (Chamberlain, 1970).

On the basis of these and previous findings (Sellers & Parker, 1969; Sellers, 1971), the following hypothesis for airborne spread of foot-and-mouth disease is put forward. Infected animals excrete virus into the air, pigs excreting the most, followed by cattle and sheep. The size of particle associated with infectivity ranges from > 6 to $< 3\ \mu$m. If the relative humidity is less than 60 % (Barlow, 1972; Donaldson, 1972), inactivation takes place and spread would be only over short

distances, i.e. metres. If the relative humidity is higher than 60% and in the absence of sunshine and pollutants, the infective particles would be expected to survive for hours, especially the larger ones (Norris & Harper, 1970; Benbough & Hood, 1971). In calm, the larger particles would sediment; in wind they would be carried long or short distances, depending on the speed of the wind and the nature of the surface over which they travel. Under certain conditions (transport over sea or as lee waves over land – Tinline, 1970), the concentration of the larger particles apparently remains the same. Deposition of the larger particles would take place in heavy rain and they would either initiate infection by aerosols from the splash on impaction or be washed away. Light rain would have no effect on the larger particles, but in such conditions and in the absence of rain the larger particles would either sediment or be blown down by wind to a level where they would be inhaled by susceptible animals.

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