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The survival of foot-and-mouth disease virus in open air conditions

By A. I. DONALDSON and N. P. FERRIS
Animal Virus Research Institute, Pirbright, Woking, Surrey

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SUMMARY

The influence of the Open Air Factor (OAF) and daylight on the survival of foot-and-mouth disease (FMD) virus held as captured aerosols on spider microthreads has been investigated. Virus inactivation due to OAF was slight. Similarly, the effect of daylight on the survival of virus was not marked. The results are discussed in relation to the airborne spread of FMD virus in nature.

INTRODUCTION

There is considerable epizootiological and experimental evidence which suggests that under certain favourable conditions the airborne spread of foot-and-mouth disease (FMD) has occurred. This evidence has been reviewed and an hypothesis for the spread of the disease by wind has been put forward by Sellers *et al.* (1973). Fundamental to this concept of long-distance transmission is that the virus, while airborne, should survive the prevailing climatic conditions. Studies on the survival of a variety of strains of FMD virus in the airborne state have shown that the relative humidity (RH) and the nature of the suspending fluid from which the virus aerosol arises may greatly affect survival (Barlow, 1972*a*, *b*; Donaldson, 1972; Barlow & Donaldson, 1973; Donaldson, 1973). However, these studies were carried out indoors within enclosed vessels and the survival of virus under such conditions may be very different from that outdoors, where the action of the Open Air Factor (May, Druett & Packman, 1969) and also sunlight may have considerable influence. Because of these considerations we have investigated the survival of FMD virus (type O_1, strain BFS 1860) held as captured aerosols on microthreads in the open air.

MATERIALS AND METHODS

Virus

FMD virus type O_1, strain BFS 1860, was used at the third bovine passage level. For the preparation of virus stocks two Devon steers were inoculated intradermolingually at about 20 sites with virus diluted 1/10 in 0·04 M phosphate buffered saline, pH 7·2 (PBS). At 24 hr. after inoculation, vesiculated tongue epithelial tissue was collected from the steers and stock preparations of virus were made by grinding the tissue in Eagle's medium. To reduce the likelihood of indirect light

effects on virus survival, the Eagle's medium did not contain phenol red. The homogenates were clarified by centrifugation at 10,000 *g* for 30 min. at 5° C., subdivided into 5 ml. samples and stored at −70° C.

Viral assay

Plaque assays were made in bovine thyroid cells (Snowdon, 1966). Three to five replicate 6 cm. Petri dishes were used per virus dilution and the cells were overlaid with Leibovitz's L-15 medium containing 0·75 % agarose and 1 % bovine serum. Staining with neutral red was carried out after about 28 hr. incubation at 37° C.

Bacteria and bacterial assay

Bacillus globigii (*B. subtilis* var. *niger*) spores (BG) were used as a physical tracer and *Escherichia coli* MRE strain 162 as a reference indicator of open air factor (OAF). Details of the preparation of stock cultures of these micro-organisms and their assay have been given previously (Donaldson & Ferris, 1974).

Aerosol apparatus and test procedures

Collison sprays with three spray-head jets (May, 1973) were used to produce aerosols. Spray suspensions consisted of 9·0 ml. of stock virus and 0·15 ml. each of concentrated *E. coli* and heated (60 °C. for 1 hr.) BG spores. The microthread technique of May & Druett (1968) was used to expose captured aerosols to outdoor conditions. Aerosol clouds were passed from a mobile Henderson apparatus (Druett, 1969) at 70 % RH through each of two 'sows' containing 13 microthread frames for 5 min. at 11 l./min. The 'sows' were rinsed with air at the same RH for 30 sec., closed off and then placed in a safety cabinet incorporating the features described by Druett & Hood (1973). The cabinet had the additional feature of a quartz glass panel 30 cm × 15 cm × 5 mm thick built into one end. Inside the cabinet and immediately behind the quartz panel there was an adjustable platform which had slots for both microthread frames and an ultraviolet (U.V.) light sensor (Blak-Ray Ultraviolet Intensity Meter, Shandon Southern Instruments Ltd, Frimley Road, Camberley, Surrey). This sensor is designed to measure light in the range from 3000 to 4000 Å with the main energy peak at approximately 3650 Å. Once loaded, the safety cabinet was moved to a shaded outside site within the confines of the Institute and, with the cabinet air flow switched on, the microthread frames were rapidly transferred from the 'sows' to the outside air exposure ducting in the floor of the cabinet. Outside air was then drawn over the frames at a speed of 2 m./sec. The details of these procedures have been previously described (Donaldson & Ferris, 1974). The influence of daylight was investigated as follows: two 'sows' loaded with 13 microthread frames each were placed in the safety cabinet and the internal RH was raised to about 80 % by spraying water from a DeVilbiss no. 152 spray attached to a pressurized can (Rals Laboratories, 480 West Aurora Road, Northfield, Ohio 44067, U.S.A.). The cabinet was moved outside and placed with the quartz panel in the direction of the sun. At time zero two groups of four frames were taken from each of the 'sows' and pushed through the insert ports in the floor of the cabinet into microthread sampling cells containing

Table 1. *The distribution of BG spores in aerosol particles used to charge microthreads*

	Size limit of particles impacted (μm.)	Total organisms recovered (%)	
		Test 1	Test 2
Stage 1	6·0–20·0	0·2	0·1
Stage 2	2·2–6·0	0·9	0·6
Stage 3	1·0–3·0	27·8	21·1
Stage 4	0·5–1·5	71·1	78·2

4 ml. of sampling fluid (PBS + 0·25 % bovine serum albumin). The remaining nine frames in one 'sow' were placed on the platform behind the quartz panel while the nine frames in the other 'sow' were left as unexposed controls. At 10 min. intervals from time zero, groups of three frames were taken from the daylight exposure platform and also from the 'sow' containing the non-exposed frames and pushed through the cabinet floor into sampling cells. During the exposure period of 30 min. air was not drawn through the cabinet but the adjustable platform holding the microthread frames and the cabinet were moved so that the light intensity registered by the sensor was maximal. The RH within the cabinet was maintained by spraying water from the atomizer as required. For the OAF experiments the survival of virus and *E. coli* was recorded as percentage viability, recoveries from the controls at time zero having been given the nominal value of 100 % (May & Druett, 1968). Recoveries after daylight exposure were also recorded as percentage viability though in these experiments the recoveries from the non-exposed control samples at each time interval were given the nominal value of 100 %. The experiments on OAF were carried out in the autumn and winter and the daylight experiments were undertaken in the spring and summer. All the experiments were done between 09.30 and 11.00 hr.

Particle sizing

Aerosols were sampled for BG spores 1 sec. after generation, using a Casella cascade impactor (C. F. Casella & Co. Ltd, Regent House, Britannia Walk, London, N.1.) as described by May (1945). Clouds were sampled for 30 sec. at 70 % RH and at a flow rate of 17.5 l./min. onto glass disks coated with 5 % (w/v) gelatin in 10% glycerol. The spores were rinsed off the disks with PBS at 40° C. for assaying.

RESULTS

The size distribution of the particles in the aerosols used to charge the microthreads was determined by assessing the proportion of BG spores trapped on the four stages of the Casella cascade impactor. The results obtained are shown in Table 1.

The majority of the particles were in the 0·5–3·0 μm. size range. The plots of these results on log-probability paper gave 50 % mass median diameters of 1·1 and 0·9 μm. for tests 1 and 2 respectively.

Table 2. *Survival of FMD virus (O_1 BFS 1860) on microthreads exposed to outside air*

Expt.	RH (%)	Temp. (°C.)	Wind direction	Wind force	Organism	Time of exposure (min.) 20	40	60
1	70	8	270–315°	Light	FMD	100*	100*	91*
					EC†	51	19	8
2	67	12	315–360°	Light	FMD	36	40	16
					EC	254	99	25
3	63	9	225–270°	Light	FMD	43	62	73
					EC	92	77	42
4	61	11	260–300°	Moderate	FMD	100	98	82
					EC	115	52	23
5	87	1	300–320°	Calm	FMD	163	123	177
					EC	71	74	58
6	86	10	260–290°	Light	FMD	104	99	36
					EC	164	121	48
7	89	7	170–320°	Light	FMD	147	128	88
					EC	82	62	36
8	75	6	260–280°	Light	FMD	45	51	37
					EC	131	115	69
9	95	7	170–230°	Strong	FMD	100	64	82
					EC	106	53	29
10	69	8	220–265°	Fresh	FMD	100	96	32
					EC	75	56	17

* Mean percentage recoveries from six microthread frames.
† *E. coli* MRE 162 included as a reference indicator of outside air factor activity.

No decay of virus occurred on microthreads in enclosed sows charged with aerosols at 70 % RH and held at indoor ambient temperature (20–22° C.) over a 60 min. period. Under the same conditions the mean decay rate for *E. coli* MRE was 0·3 %/min.

Out of doors high recoveries of FMD virus were obtained after 60 min. exposure (Table 2). For seven of the ten tests carried out *E. coli* did not survive as well as FMD virus but on the other three occasions *E. coli* showed slightly higher survivals. In order to avoid the inactivating effects of mid-range and low RH on FMD virus, experiments were undertaken only when the outside RH was above 60 %. The loss of *E. coli* which occurred could not be correlated with wind coming from any particular compass bearing. For most of the experiments the wind was blowing from the west or north-west.

FMD virus was found to be much more stable in daylight than *E. coli*. In strong sunlight *E. coli* was rapidly inactivated but FMD virus survived comparatively well (Table 3). The correlation for both micro-organisms between the amount of inactivation which occurred and the mean intensity of U.V. light during the exposure periods was weak. One factor possibly contributing to this was the considerable fluctuation of light intensity due to the movement of clouds across the sun on bright sunny days or the occasional periods of bright sunshine which occurred between gaps in the clouds on overcast days. The mean of these light inten-

Table 3. *Survival of FMD virus (O_1 BFS 1860) on microthreads exposed to daylight*

Expt.	Temp. (°C.)	RH (%)	u.v. intensity (μW./cm^2 × 100)	Organism	Time of exposure (min.) 0	10	20	30
1	21	76	1·0	FMD	124*	202*	179*	132*
				EC	125	108	57	35
2	27	86	21·5	FMD	40	193	9	110
				EC	55	21	16	9
3	25	81	15·5	FMD	104	129	23	5
				EC	92	67	2	0·5
4	26	80	1·8	FMD	54	57	57	22
				EC	73	41	22	2
5	26	81	3·2	FMD	110	148	81	73
				EC	61	33	3	0·5
6	26	82	3·9	FMD	122	59	24	24
				EC	104	38	11	3
7	23	81	0·4	FMD	108	109	72	61
				EC	83	72	44	12
8	24	90	14·0	FMD	119	73	19	13
				EC	117	19	4	0·1
9	23	86	6·6	FMD	98	97	70	51
				EC	103	57	11	2
10	21	84	16·5	FMD	124	148	31	33
				EC	125	71	7	0·5

* Mean percentage recoveries from eight microthread frames at time zero and three microthread frames for other exposure periods.

sities therefore gave only an approximation; it would have been preferable if it had been possible to record the total u.v. over 30 min.

DISCUSSION

The aim of the present investigation was to examine the effect of outside air and sunlight on the airborne survival of FMD virus. The results show that, in the case of outside air, the effect on O_1 BFS 1860 virus survival was slight. *E. coli* MRE 162 also survived comparatively well in outside air. The observation that the inactivation rates for the two micro-organisms were independently variable in all of the experiments supports the suggestion by Benbough & Hood (1971) that different pollutants may account for the viricidal and bactericidal activities of outside air.

The choice of autumn and winter for the investigation was deliberate since this is the time of year when the airborne spread of FMD more usually occurs in temperate countries (Fogedby, Malmquist, Osteen & Johnson, 1960; Hurst, 1968; Smith & Hugh-Jones, 1969; Sellers & Forman, 1973; Primault, 1974). Autumn evenings are the occasions on which temperature inversions tend to occur in the U.K. so that it is likely that higher levels of OAF than observed in the present study may occur. It is also probable that the nature of OAF will differ in different places (Druett, 1973).

Using Pasquill's (1961) formula and assuming an RH of above 60% and lack of inactivation from other causes, it has been calculated (Sellers & Parker, 1969; Sellers, 1971) that a dose of FMD virus large enough to infect susceptible animals could travel up to 50 km. downwind of the source. Benbough & Hood (1971) found that Semliki Forest virus and T coliphages were inactivated at a considerably enhanced rate in open air compared with enclosed air and they therefore suggested that the calculations for the long distance spread of FMD virus might need considerable alteration. But if the influence of OAF on FMD virus survival is of the magnitude indicated by the present investigation, only slight adjustment of the calculations is required.

The effect of light on FMD virus was first examined by Perdrau & Todd (1933). They showed that FMD virus could be photosensitized if methylene blue was added to virus suspensions and that, compared with the viruses of vaccinia, herpes simplex, fowl plague, louping-ill, Borna disease and canine distemper, FMD virus was photoresistant. The photoresistance of FMD virus to both daylight of normal laboratory intensity and also artificial light was confirmed by Skinner & Bradish (1954). Suspensions of the viruses of vesicular stomatitis, influenza, Newcastle disease and fowl plague were strongly inactivated by exposure to daylight for 4 hr., but FMD was stable. The exact wavelengths which produced the inactivation were not determined but the authors were of the opinion that U.V. was not a major factor. Appleyard (1967) reported that daylight produced inactivation of Sindbis, Murray Valley encephalitis, influenza and rabbitpox viruses but not poliovirus over a period of 4 hr. The wavelength action spectrum was in the 3300–4700 Å. range, i.e. long-wave U.V. and visible-light ranges. From these and other published findings Appleyard concluded that there might be a correlation between virus group and photo-sensitivity; arboviruses and myxoviruses appeared to be photosensitive, picornaviruses photoresistant and pox viruses of intermediate photosensitivity. The precise mechanism for the photoinactivation of viruses by daylight has yet to be determined. It has been proposed by Appleyard (1967) that inactivation results from photo-oxidation of the sensitized viral nucleic acid, the sensitizing agent being either an essential viral component or adsorbed or incorporated extraneous material.

In the present study and also those previously described, the general photoresistance of FMD virus has been demonstrated. However, during outbreaks of disease, aerosols of FMD virus may arise from a variety of sources, e.g. from infected animals, particularly pigs, but also from the splashing of milk in dairies and the spray disposal of slurry (Sellers, 1971). Both in size and composition, these infectious aerosol particles will obviously vary considerably, and since some naturally occurring substances, e.g. serum and cysteine, have been shown to protect viruses against photoinactivation, whereas other substances, e.g. riboflavin and vitamin A, can sensitize (Appleyard, 1967; Skinner & Bradish, 1954), this makes it difficult to predict the extent to which daylight will influence virus survival in all cases. These considerations are likely to be even further complicated by the influence of other variables such as the relative humidity and the temperature.

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