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Air sampling of smallpox virus

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SUMMARY

Airborne smallpox virus has been recovered in an isolation hospital using an adhesive surface sampling technique in the presence of very low aerosol concentrations. Previous work in this field is reviewed. Successful recovery of airborne virus depends on sampling large volumes of air with a suitable sampler and thorough investigation of the whole sample taken for the presence of viable virus. More information on the characteristics and behaviour of airborne smallpox virus is needed in particular with regard to the future design and siting of smallpox isolation units.

INTRODUCTION

It is generally accepted that a patient with smallpox becomes infectious when the first external signs of disease appear. Virus is usually plentiful in the respiratory and oral secretions (Downie *et al.* 1961) and also in skin lesions. Smallpox is most frequently transmitted in the early stages of the disease during close contact but infection may also occur through handling infected clothing and bedding (Cramb, 1951; Dixon, 1962). Outbreaks of smallpox have occurred when contact with known infected individuals or families could not be established (Power, 1882; Barry, 1889; Peirce, Melville, Downie & Duckworth, 1958; Perkins & Vaughan, 1961; Dixon, 1962; B.M.J., 1970). There is evidence which suggests that dissemination of infection to remote parts of a hospital may have occurred by airborne transmission (WHO, 1970). Accidental laboratory infection with smallpox is rare but a recent incident, which gave rise to two fatal secondary cases, appears to have occurred through exposure to an infective aerosol.

Immunization of the general population is no longer encouraged, largely because of the incidence of serious side effects, but the introduction of smallpox through air travel is an ever present possibility despite world-wide attempts to eradicate the disease (Henderson, 1973).

The study of the behaviour of airborne smallpox virus has not received much attention and there are few references in the literature (Meiklejohn *et al.* 1961; Downie *et al.* 1965). Despite the plentiful presence of smallpox virus in oral secretions, skin lesions and contaminated bedding the reported air sampling results appeared to indicate low air concentrations of virus. Rabbitpox virus has been successfully sampled by an adhesive surface sampling technique (Thomas, 1970*a*, *b*)

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and it is the application of this method to the sampling of airborne smallpox virus arising from a human source which is reported in this paper.

MATERIALS AND METHODS

Samplers

Slit sampler (Casella) using plates coated with S.G.B. mixture (sucrose, glycerol, and bovine serum albumin; Thomas, 1970*a*), Porton impingers (May & Harper, 1957) containing 10 ml. of '199' medium with antifoam and sedimentation plates coated with S.G.B. mixture were used.

Virus culture

A HeLa cell line (Appleyard & Westwood, 1964) was used for preliminary culture of virus recovered in S.G.B. plates (Thomas, 1970*a*). The cells were harvested after 7 days, disrupted by freezing and thawing and then inoculated in 0·4 ml. amounts into 11-day-old embryonated hen's eggs (5 eggs for each plate). Pirsch & Purlsan (1962) have described hyperplastic foci in HeLa cell monolayers caused by variola virus. The monolayers in the experiments described here were examined before harvesting for evidence of cytopathic effects but none of the changes seen were considered sufficiently well characterized to indicate variola virus. In addition air sampling collected particles of many types some of which may have caused changes in the HeLa cell monolayers which were in no way related to variola virus replication. Impinger samples were inoculated in 0·2 ml. volumes into hen's eggs. All the eggs were incubated for 5 days before being examined for the presence of pocks. Any membranes which showed any changes suggestive of pock formation after incubation were passaged a second time.

Air sampling procedures

Air sampling was carried out for 2 days in two wards (male and female) of a smallpox isolation hospital at the end of a variola minor outbreak. The female ward contained three adult patients only one of whom still had a few dry lesions ('seeds') which were slow in separating. In the male ward were two children awaiting discharge and an adult male who still had a number of active lesions which were considered to be secondarily infected. Air samplers were used within a few feet of the occupied beds in both wards but the sedimentation plates were exposed in groups approximately 20 ft. away from these beds. Sampling periods of $\frac{1}{2}$ to 1 hr. per plate were used with the slit sampler operating at a flow rate of 1 ft.^{3}/min. while the sedimentation plates were exposed for many hours at a time. The impinger samplers were operated at a flow rate of 11·5 l./min. for 15 min. per sample. A summary of the sampling procedures is given in Table 1.

Table 1. *Summary of air sampling procedures undertaken in the male and female wards of the smallpox isolation hospital*

Sampler	Ward	Day	No. of samples	Period of sampling (hr.)	Volume of air sampled ft.³	Volume of air sampled l.
Slit sampler	Male	1	9	8·25	495	14,008·5
		2	14	7·5	435	12,310·5
Slit sampler	Female	1	10	9·25	555	15,706·5
		2	2	1	60	1,698
Impingers	Male	2	6	1·5	36·6	1,035
Impingers	Female	2	4	1	24·4	690
Sedimentation plates	Male	1, 2	11	36	—	—
Sedimentation plates	Female	1, 2	7	39·25	—	—

Table 2. *Detection of variola virus in the samples taken with the slit sampler in the male ward*

Day	Sample no.	No. of eggs (5 eggs/sample) showing variola pocks: First passage	Second passage
1	1	1	0
	2	2	5, 5
	3	3	5, 5
	4	0	—
	5	1	0
	6	0	—
	7	0	—
	8	3	5, 5, 5
	9	4	5, 5, 5, 5
2	1	0	—
	2	1	0
	3	0	—
	4	0	—
	5	2	5, 5
	6	2	5, 5
	7	0	—
	8	2	5, 5
	9	2	5, 5
	10	0	—
	11	0	—
	12	3	0, 0, 0
	13	3	5, 5, 5
	14	2	5, 5

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Table 3. *Detection of variola virus in the samples taken with the slit sampler in the female ward*

Day	Sample no.	No. of eggs (5 eggs/sample) showing variola pocks: First passage	Second passage
1	1	2	0, 0
	2	0	—
	3	3	0, 0, 0
	4	2	0, 0
	5	2	0, 0
	6	2	0, 0
2	1	0	—
	2	1	0
	3	1	0
	4	1	0
	5	0	—
	6	0	—

Table 4. *Detection of variola virus in sedimentation plate samples taken in the male ward*

Period exposed	Sample no.	No. of eggs (5 eggs/sample) showing variola pocks: First passage	Second passage
11.45 to 19.45	1	2	0, 0
	2	1	0
19.00 to 07.00	3	4	5, 5, 5, 5
	4	2	0, 0
	5	3	5, 5, 5
12.00 to 20.00	6	2	5, 5
	7	0	—
	8	1	0
	9	0	—
	10	2	0, 0
	11	0	—
	12	1	0

RESULTS

Slit sampler

Pock formation characteristic of smallpox virus was seen only in samples taken in the male ward where 10 of the original air samples taken with the slit sampler were shown to have contained smallpox virus. None of the samples in the female ward were positive. The results of these investigations are given in Tables 2 and 3.

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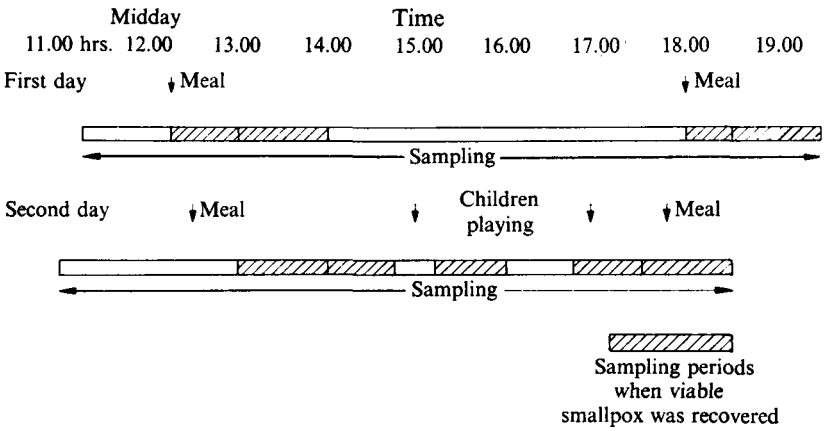

Fig. 1. The diagram indicates the periods when smallpox virus was detected in the air samples taken with the slit sampler in the male ward.

Sedimentation plates

Eleven plates were exposed in the male ward and seven plates in the female ward during the two days of sampling. Again only the male ward samples were positive and the results are given in Table 4.

Impinger samplers

Six samples were taken in the male ward and four in the female ward. No evidence of smallpox was found in any of these samples.

DISCUSSION

Airborne variola virus was detected in the slit sampler plates and in the sedimentation plates only in the male ward. At the time of sampling the adult male patient alone was considered to be infectious since he still had a large number of skin lesions which were separating slowly. Many appeared to be secondarily infected with consequent breakdown of the lesions. On both days successful recovery of airborne virus with the slit sampler occurred during very similar periods which coincided with the main time of activity in the wards. It is the movements of the adult male patient which can best be related to the air sampling results. He did not rise for breakfast but did so just before lunch, about 12.20 p.m. when his bed was made, and similarly in the late afternoon for the next meal. On both days it can be seen in Fig. 1 that airborne virus was recovered during these two similar periods.

The two male child patients were rather subdued on the first day but on the second day in the afternoon they indulged in a wild romp which involved leaping on and off the bed of the adult male. It is possible that this additional activity gave rise to the positive samples obtained in the middle of the afternoon of the second day (Fig. 1).

The results of air sampling with the slit sampler in the male ward given in

Table 2 are based on unequivocal variola pock formation in the second egg passage. It is not possible to place any reliable quantitative interpretation on these results, only the presence or absence of viable airborne virus.

Table 4 shows that there were two positive sedimentation plates out of the three exposed overnight in the male ward and one positive sample was obtained during the daytime exposures. The results are too few to place any useful interpretation on the findings, other than that viable airborne virus was carried well down the ward. The plates were deliberately placed some distance away from the possible source of virus since fall-out of infected particles at close range would be certain to occur as has already been observed by other workers (Downie *et al.* 1965). The exposure of many more plates would have been helpful but the demands of continuous air sampling limited the numbers of plates available for use in this way.

The impinger samples were all negative. One was taken during the period when successful recovery occurred with the slit sampler. Because of the very small quantity of airborne virus it is probable that the 15 min. period of sampling for the individual impingers was too brief. In addition, only a tenth of the total volume of the sampling fluid was tested. Westwood, Boulter, Bowen & Maber (1966), sampling for rabbitpox with impingers also failed to recover any viable virus. Downie *et al.* (1965) were successful in collecting airborne smallpox using impingers. The number of patients and consequently the concentration of airborne virus however was considerably greater than that present in the isolation hospital where the air sampling described in this paper was carried out.

Meiklejohn *et al.* (1961) carried out sampling for airborne smallpox virus with small glass funnels containing tightly packed dry cotton wool. Thirty-eight samples were taken, many in close proximity to acutely ill patients. During several sampling periods agitation of bed clothes and vigorous sweeping of floors was carried out to create an aerosol. Only one of the samples proved positive, despite the large volumes of air sampled (4500–9000 l. of air/sample). This method of sampling is not very efficient for collecting small particle sizes in the respirable range, i.e. those less than 10 μm. in size. The attempts that were made to collect particles (droplet nuclei) expelled from the mouths of patients with oral lesions would not be likely to be successful (W.H.O., 1964) while the likelihood of survival of virus in any such particles collected on the cotton wool would be diminished by the drying effect (Lovelock, Porterfield, Roden & Sommerville, 1952).

Downie *et al.* (1965) reported air sampling for smallpox virus under conditions very similar to those described by Meiklejohn *et al.* Porton impingers, sedimentation (settle) plates and the top stage of an Andersen sampler (Andersen, 1958) were used. Virus was recovered with the first two types of sampler but none with the third which was functioning in this case as a simple sieve sampler with a very low collection efficiency. The presence of virus in the air samples was investigated by inoculating small volumes into embryonated hen's eggs without preliminary concentration. Of the impinger samples 11 %, and of the sedimentation plate samples 40 % were positive. Large droplets expelled from patients' mouths would fall out quickly into the sedimentation plates but only a proportion of the much

smaller airborne droplets would be collected by the impinger. Tyler & Shipe (1959) found the impinger as efficient as other samplers for fine relatively monodisperse aerosols but for heterogeneous airborne particles, such as those in natural aerosols, other types of sampler were more efficient.

Downie and his colleagues also examined the levels of airborne virus arising from bedding contaminated by skin lesions and in this part of the investigation 55% of the air samples were positive, mainly the sedimentation plates. Bed clothes contaminated with discharges from skin lesions provide a much larger source for the dispersal of virus.

The results for air sampling given in the papers by Meiklejohn *et al.* (1961) and Downie *et al.* (1965) reviewed above appear to indicate that despite the very plentiful presence of smallpox virus in oral secretions, skin lesions and contaminated bedding, concentrations of airborne virus close to the patients were not particularly high and that most of the particles carrying smallpox virus tended to sediment rapidly. The slit sampler used in the author's investigations has a high collection efficiency for particles of 1 μm. and above, superior to that of the cotton wool filter used by Meiklejohn *et al.*, and more suitable for sampling heterogeneous natural aerosols than the impingers used by Downie *et al.* The adhesive surface sampling technique has the added advantage of obtaining preliminary replication in HeLa cells before egg inoculation. In this method all the particles carrying viable virus collected in the sample are brought into contact with susceptible tissue cells without further need for manipulation. The method was assessed before use by examining dilutions (up to 10^{-8}) of suspensions of smallpox virus. Virus was not detectable by direct inoculations into eggs at 10^{-6}, 10^{-7} and 10^{-8} dilutions but was readily demonstrated in eggs after preliminary passage of these dilutions in HeLa cells. The combination of an efficient sampler and a sensitive detection method enabled the recovery of viable smallpox virus from very low airborne concentrations.

Hospitals for the isolation of smallpox patients in the past were usually sited in the country away from centres of population. Many of these hospitals are now very old and are in need of replacement. There has been a move in recent years to site new isolation units adjacent to general hospitals, sometimes in areas of high population. These units require extensive and complex air filtration systems to ensure containment of airborne infectious material. The author has undertaken commissioning trials of one such isolation unit. The results of tests on the air filtration system, coupled with the slowly growing body of knowledge concerning the characteristics and behaviour of airborne smallpox virus, clearly indicated a need for very careful siting, construction, and maintenance of the units together with periodic tests of the integrity and efficiency of the air filtration systems.

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