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Sampling rabbit pox aerosols of natural origin

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SUMMARY

Natural aerosols of rabbit pox virus produced by groups of infected rabbits were sampled with a slit and Andersen sampler using an adhesive surface sampling technique (Thomas, 1970). The higher rate of successful recoveries of airborne virus compared with a previous report is believed to be due to the much larger volume of air sampled by this technique and to the processing of the whole of the sample collected by the direct addition of a cell suspension to demonstrate the presence of viable virus.

INTRODUCTION

Natural aerosols of the pox viruses have been sampled with varying degrees of success (Meiklejohn *et al.* 1961; Downie *et al.* 1965; Westwood, Boulter, Bowen & Maber, 1966). Sampling methods used included settle plates, cotton wool filters, sieve samplers, impingers and an electrostatic precipitator (Morris, Darlow, Peel & Wright, 1961). The opportunity arose of sampling natural rabbit pox aerosols under the same conditions as those reported by Westwood *et al.* (1966) with groups of animals infected with rabbit pox. Air samples were taken using an adhesive surface sampling method which had proved effective with artificial aerosols of vaccinia virus (Thomas, 1970).

MATERIALS AND METHODS

Samplers. Slit sampler (Casella) and an automated version. Andersen sampler (Andersen, 1958).

Cell culture. HeLa cells (Appleyard & Westwood, 1964). Monolayers prepared from $7–8 \times 10^6$ cells, incubated at 37° C. under 5% CO_2 for 2 days.

Nutrient medium. '199', with neomycin (70 units/ml.), amphotericin B (0·0025 mg./ml.), calf serum 5%.

Rabbit pox virus. Utrecht strain.

Rabbits. Mixed sexes, about 2·5 kg.

Infection method. Respiratory route (Westwood *et al.* 1966).

Sampling methods. Petri dishes were coated with 0·2 ml. of either 10% calf serum, 10% bovine serum albumin or a mixture of equal volumes of saturated sucrose solution and glycerol with 0·1% of 10% bovine serum albumin (S.G.B. mixture, Thomas, 1970). Sampling periods of 1 hr. were used. A HeLa cell suspension was then added to each plate to form a monolayer in direct contact with the aerosol particles that had been collected. Plaque counts were taken after 2 days.

RESULTS

Three experiments were carried out with different groups of rabbits.

Experiment 1

Air samples were taken with a slit sampler from a room containing five infected rabbits via a 1 in. diameter tube passing through a hole in a door. Ten per cent calf serum was used as the adhesive sampling surface and six consecutive 1-hr. samples were taken on the 5th day after infection. Only one animal had a slight nasal discharge at this time and no other signs of infection appeared. The plaque counts obtained from the samples are given in Table 1.

Table 1. *Expt* 1, *Sampling natural rabbit pox aerosol using* 10% *calf serum as an adhesive surface*

Sample no.	Plaque counts	Vol. of air/ sample (cu.ft.)
1	2	60
2	5	
3	1 (p.m.)	
4	7	
5	10	
6	3	

p.m., Poor monolayer.

Experiment 2

Ten per cent bovine serum albumin was used to coat the plates. Sampling was carried out as described in the first experiment, the room containing 30 infected rabbits. Groups of consecutive 1-hr. samples were taken over a period of 7 days commencing on the 4th day after infection. Plaque counts are given in Table 2. An Andersen sampler was used for one sample on the 8th day after infection. Glass disks coated with 10% bovine serum albumin replaced the agar in the sampling dishes. The disks were of the right thickness to establish the correct distance between the under surface of the orifice plates and the collecting surfaces. Table 3 gives the plaque counts obtained with the Andersen sampler.

Experiment 3

In this investigation samplers were placed inside the room holding the rabbits on the 8th day after infection. There were 33 animals, 12 with rashes and seven had discharges. The slit sampler and the automated slit sampler were used with the sample plates coated with the S.G.B. mixture. Five consecutive 1-hr. samples were taken in parallel with the two samplers and the resulting plaque counts are given in Table 4.

DISCUSSION

The same room was used for all three experiments and the general conditions, temperature, humidity, ventilation, feeding and cleaning routines, sampling periods, etc., were similar. Approximately six air changes an hour took place

continuously causing fairly rapid dilution of the aerosols produced by the animals. The cubic capacity of the room was approximately 4500 cu.ft.

In the first experiment 10% calf serum was used to coat the sampling dishes. It is likely that efficient sampling occurred only during the first 20 min. each plate

Table 2. *Expt 2, Sampling natural rabbit pox aerosol using 10% bovine serum albumin as an adhesive surface*

Sample no.	Plaque count	Vol. of air/sample (cu.ft.)	Days after infection	Number of rabbits			
				Live	With		Dead
					Discharges	Rash	
1	0						
2	0						
3	0						
4	0	60	4	30	0	0	0
5	0						
6	0						
7	6						
8	0	60	5	30	2	5	0
9	1						
10	1						
11	1						
12	1	60	7	30	5	12	0
13	1						
14	3						
15	2						
16	7						
17A	86						
18	2	60	8	29	10	15	1
19	2						
20 p.m.	0						
21	25	60	—	—	—	—	—
22	12						
23	74	120	**9**	**27**	**9**	**15**	**2**
24	1						
25	1	60	—	—	—	—	—
26	1						
27	13						
28 p.m.	0						
29	81						
30	13	60	10	25	10	18	2
31 p.m.	0						
32	4						
33	1						

Sample 17A, Andersen sampler—plaque count is total of plaques on all stages of the sampler. p.m., Poor monolayer.

was used since the calf serum dries out to give a smooth non-adhesive surface. Five animals were used in this first experiment and of these only one had evidence of infection so that the amount of viable airborne virus produced was probably small. Despite these limiting factors all the samples were positive.

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The bovine serum albumin used in the second experiment behaves similarly to the calf serum. It was considered to have a marginal advantage in not containing the inhibitory factors sometimes present in calf serum. The single sample taken on the 5th day after infection was negative despite the presence of two animals with discharges. Both were on the far side of the room away from the sampling orifice in the door, while in the first experiment the cages were placed as close as possible to the mouth of the sampling tube.

Table 3. *Expt* 2, *Sampling natural rabbit pox aerosol using an Andersen sampler and* 10% *bovine serum albumin as an adhesive surface*

Stage no.	Plaque count	Distribution (%)	Total vol. of air sampled (cu.ft.)
1	25	29	
2	17	20	
3	24	28	60
4	15	17	
5	4	5	
6	1	1	

Table 4. *Expt* 3, *Sampling of rabbit pox aerosol with a standard and an automated slit sampler, using S.G.B. mixture as the sampling medium*

Sample no.	Plaque counts: Slit sampler	Plaque counts: Automated sampler	Vol. of air/sample (cu.ft.)
1	17	6	
2	24	14	
3	16	5	60
4	6	28	
5	16	17	
Totals	79	70	300

The samples taken on the 7th, 8th and 9th days after infection showed increasing total plaque counts in keeping with the progress of the disease in the animals. Where virus recoveries were higher in the mornings this was probably due to feeding and cleaning.

The results of previous investigations with the adhesive surface sampling technique (Thomas, 1970) suggest that the individual plaques in the samples taken with the slit and Andersen samplers represent separate aerosol particles carrying virus. In the Andersen samples taken on the 8th day viable virus was mainly carried on the larger particles collected in the upper four stages of the sampler, i.e. $> 2{\cdot}5\,\mu$.

For the third experiment the plates were coated with the S.G.B. mixture which remained fully adhesive throughout the sampling period. The samples were taken in the afternoon of the 8th day after infection when feeding and cleaning had been completed. This had been a period of low plaque counts in the second

experiment, and the higher counts obtained during this time in the third experiment are due in large part to sampling inside the room and the superior collection efficiency of the S.G.B. mixture. It remained adhesive for the 60 min. exposure in the sampler while the calf serum and bovine serum albumin dried out to give a smooth, non-adhesive surface. Impaction on such a surface has been shown to result in loss of small particles due to break up of aggregates or whole particles on impact (Davies, Aylward & Lacey, 1951). Many of the fragments would not be retained by a non-adhesive surface but be swept on by the air stream.

Table 4 shows considerable variation in the plaque counts for simultaneous samples taken by the two slit samplers. However, the total counts for the whole sampling period were very close, 79 and 70. The automated slit sampler was a prototype version which enabled samples to be taken at chosen intervals for preset periods at 1 cu.ft. of air/minute. Its sampling efficiency was closely similar to the standard slit sampler. In this experiment the machine was set to take five 1-hr. samples consecutively while the standard sampler was serviced manually at 1-hr. intervals.

When poor monolayer formation occurs, and it is not possible to make a reliable plaque count, the contents of the sample plate can be subcultured onto another monolayer and viable virus detected. The plaque counts in these circumstances, however, would not be related to particles collected.

Westwood *et al.* (1966) reported experiments in air sampling for rabbit pox virus which were undertaken in the same rooms and under conditions similar to those in the first and second investigations reported above. Sampling was carried out with raised glass impingers (May & Harper, 1957) and an electrostatic precipitator (Morris *et al.* 1961). Morris has shown (unpublished) that the impinger and the precipitator have similar sampling efficiencies in the range down to 1 μ size particles, but the precipitator has a superior collection efficiency for submicron particles. A series of samples were taken over the period between the 3rd and 12th days after infection inclusive. Most of the samples were obtained with the precipitator but some parallel samples were taken with impingers. The latter were operated for 15 min. each sample at 10 l./min., while the precipitator was run at the same rate for 30 min./sample. Airborne virus was recovered only with the electrostatic precipitator on the 6th and 7th days after infection. The air sampling results reported by Westwood *et al.* (1966) were a summary of seven experiments in which the numbers of animals used varied, but in all cases exceeded the numbers used in each of the rabbit pox experiments reported above. Onset of infection and the ensuing progress of the disease in the various groups of animals used was closely similar to that observed in the experiments described above but more developed discharges.

The rabbit pox aerosol arises from the infected discharges produced by the animals. Westwood *et al.* (1966) examined these discharges and found that the virus content reached maximum levels on the 6th and 7th days after infection. This corresponded with their successful aerosol samples. But their findings also showed that these discharges continued to have a high titre of virus up to the

12th day. However, they obtained no positive air samples on the 8th to the 12th days.

The lack of success in the air sampling carried out by Westwood *et al.* (1966) was due to a number of factors. Most important was the relatively small volume of air taken in each sample and with only representative quantities of the sampling liquid inoculated into hen's eggs some virus might have been missed in this way. In addition the sampling characteristics of the impingers and the electrostatic precipitator used tended to discriminate against collection of the larger particles from a heterogeneous aerosol. The results (Table 3) of the Andersen sampler indicated a preponderance of particles larger than 5 μ carrying viable virus.

Brachman *et al.* (1964) recommended that data obtained with any specialized sampler should be correlated with results obtained with a standard impinger reference sampler. In a previous report (Thomas, 1970) comparisons were made between the impinger and the adhesive surface sampling technique using artificial virus aerosols. However, in the investigations reported above, it was decided not to use an impinger in view of the failure to recover viable airborne virus reported by Westwood *et al.* (1966) and its known particle discrimination behaviour. In addition the adhesive surface sampling technique had been developed specifically to obtain long sampling periods while the impinger sampler has a relatively short sampling period. Brachman *et al.* (1964) also recommended comparison with an Andersen sampler. In this case the adhesive surface sampling method is directly applicable to the Andersen sampler and has been shown to work successfully with this sampler both with artificial virus aerosols (Thomas, 1970) and with a natural virus aerosol as reported above.

The successful recovery of airborne virus in the three experiments described in this report is a consequence essentially of the large volumes of air sampled, coupled with the wide particle range collected by the samplers and the fact that the whole of each sample taken was processed to demonstrate viable airborne virus.

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