*J. Hyg., Camb.* (1978), **81**, 9
*Printed in Great Britain*



# Detection of airborne polyoma virus

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(*Received* 6 *September* 1977)

## SUMMARY

Polyoma virus was recovered from the air of an animal laboratory housing mice infected with the virus. Air samples were obtained by means of a high volume air sampler and further concentrated by high speed centrifugation. Total concentration of the air samples was $7 \cdot 5 \times 10^7$. Assay for polyoma virus was by mouse antibody production tests. Airborne polyoma virus was detected in four of six samples.

## INTRODUCTION

Polyoma virus (PV), a member of the papovavirus group, is frequently encountered in laboratory mice and can be oncogenic under certain conditions (Parker, Tennant & Ward, 1966). It is excreted in the urine of infected mice in copious amounts, and can be isolated from faeces and saliva (Rowe, 1961). Papovaviruses are relatively resistant to dehydration and other environmental stresses (Akers, Prato & Dubovi, 1973). PV has been recovered from bedding and dust of animal laboratories and from nests of wild mice (Rowe *et al.* 1958). We have documented airborne transmission of PV in an animal laboratory, the infection rate being influenced by ventilation, length of exposure and other experimental variables (McGarrity, Coriell & Ammen, 1976). This report describes methods to detect airborne PV. These techniques might be appropriate for detection of other airborne viruses.

## MATERIALS AND METHODS

### *Assay for polyoma virus*

Samples were assayed for PV by cell culture and development of specific antibody. Cell culture assay was by inoculation onto 3T-6 mouse embryo fibroblasts. Medium was Hanks Eagle's medium plus 10% uninactivated fetal bovine serum with 200 $\mu$g gentamicin/ml. Samples of 0·1 ml in serial dilutions ranging from $10^{-1}$ to $10^{-4}$ were added to confluent monolayers. Samples containing $10^5$ TCID50 were used as positive controls; dilutions for these ranged from $10^{-1}$ to $10^{-7}$. Negative controls consisted of uninoculated samples and samples inoculated with air or urine samples known to be negative for PV. Cultures that exhibited cytopathic effects suggestive of PV were injected intraperitoneally into strain NAL mice. Mice were housed in sterile atmospheres for 2 weeks when they were orbitally bled for the mouse antibody production (MAP) test. A minimum of four mice was used for each assay. Plasmas were assayed for specific anti-polyoma

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haemagglutination inhibition (HI) antibodies. Positive and negative controls were employed in each test.

*Animal laboratory*

Air sampling was performed in a 6 × 10 ft animal laboratory during cage changing, which took approximately 1 h. The volume of the room was 18 $m^3$ and was supplied with 15 fresh air changes/h through medium efficiency filters. The room housed 30 mouse cages; each contained three mice (strain NAL, National Animal Laboratories, Creve Couer, MO.) that were injected intraperitoneally with $10^5$ TCID50 of PV. Anti-polyoma haemagglutination inhibition (HI) tests were performed to demonstrate seroconversion by the mouse antibody production (MAP) test (Parker *et al.* 1965).

*Air sampling*

Low and high volume sampling was performed. Low volume air sampling (LVAS) was performed with all glass impingers (AGI-4) containing Hanks–Eagle's medium, 10% bovine serum, 200 μg gentamicin/ml, 0·1% thallium acetate, 0·002% Tween 80, and 0·002% anti-foam emulsion (Dow-Corning, Midland, MI.) Each AGI-4 contained 22 ml; sampling rate was 12·3 l/min. High volume air sampling (HVAS) was performed with a Litton high volume air sampler (HVAS) (Model M., Litton Systems, Minneapolis, Minn.). The HVAS monitored 1100 l of air/min and deposited suspended particulates into a small amount of liquid (5 ml/min) thereby concentrating airborne particles approximately $2{\cdot}5 \times 10^5$. Upon entering the sampling orifice, airborne particles acquired an electrical charge and were collected on an oppositely charged disk that was constantly washed with the sampling medium. Assay for PV in air samples was by the mouse antibody production (MAP) test. Four to ten mice were used for each assay. Test animals together with inoculated and uninoculated controls were housed in a mass air flow room for 3 weeks when they were orbitally bled and assayed for HI antibody specific for PV. Cross infection due to either airborne or contact transmission does not occur in this room because of the distribution of filtered air and handling techniques (McGarrity *et al.* 1976).

Medium for the HVAS was the same as that used in LVAS. In separate studies, anti-foam and/or Tween 80 were/was omitted from HVAS media. Where noted, HVAS medium was further concentrated after sampling. This consisted of low and high speed centrifugation (16000$g$, 20 min and 82600$g$ for 60 min). The high speed pellet was resuspended in T 15 NE (0·01 M Tris HCl, 0·15 M-NaCl, and 0·0002 M EDTA), and layered onto an 8 ml pad of 10% CsCl (w/w) and centrifuged at 82600$g$ for 60 min (SW 27). The pellet was resuspended in 0·01 M Tris HCl, pH 8·3; 0·1 ml was used to inject individual PV free mice I.P. for the MAP test. This procedure further concentrated the sample approximately 300 ×. Therefore, airborne particulates were concentrated a total of $7{\cdot}5 \times 10^7$ (sampling: $2{\cdot}5 \times 10^5$; concentration: $3 \times 10^2$).

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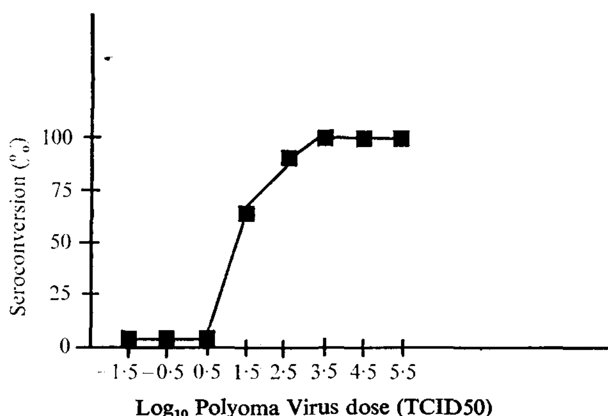


Fig. 1. Dose response of NAL mice to I.P. injection of polyoma virus.

Table 1. *Air sampling for polyoma virus**

| Procedure | No. positive/ No. air samples† |
|---|---|
| LVAS‡ | 0/12 |
| HVAS§ + Tween + anti-foam | 0/6 |
| HVAS + Concentration + Tween + anti-foam | 0/7 |
| HVAS + concentration | 4/6 |

* Samples obtained during changing cages of polyoma infected mice.
† Determined by mouse antibody production tests.
‡ LVAS, low volume air sampling.
§ HVAS, high volume air sampling.

## RESULTS

All mice injected I.P. with PV developed high titres of HI antibodies. The dose response of NAL mice to tenfold dilutions of PV is illustrated in Fig. 1. Twenty mice were used for each dilution. By extrapolation 1 TCID50 is approximately equal to 1 ID50 by I.P. inoculation. The inoculum used to inject mice in these studies ($10^{5.5}$ TCID50) was adequate to seroconvert all animals. The mean HI titre developed in response to this inoculum was 6144. Urine samples from 16 PV infected mice were separately tested for PV in the urine. The concentrations of PV in urine of infected mice varied from $10^1$ to $10^4$ TCID50 in the 3T-6 system. These isolates were identified as PV by the MAP test.

Air samples were obtained weekly. Results of relevant air samples are summarized in Table 1. PV was never recovered by LVAS. HVAS media that contained Tween 80 and anti-foam were always negative. The possible effects of Tween 80 and anti-foam, alone and in combination on cell culture propagated PV were determined by plaquing on 3T-6 mouse embryo fibroblasts. Tween 80, alone or in combination with anti-foam reduced the titre by approximately 0·5 log, from $10^{5.5}$ to $10^5$ in the 3T-6 system. Anti-foam emulsion alone had no effect. While these results were not statistically significant, subsequent sampling was

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performed without these additives. HVAS sampling media without Tween 80 and anti-foam yielded PV on four of six occasions after concentration. PV was detected from the four positives after, but not before, concentration.

Mean antibody titres in the four groups of seroconverted mice were 45 (four mice), 2560 (ten mice), 5120 (ten mice), and 5120 (ten mice). All animals in each group underwent seroconversion. Concentrated pellets that yielded positive MAP samples were simultaneously inoculated in 3T-6 cultures on two occasions. One sample (ā titre 5120 by MAP) yielded $3 \times 10^3$ TCID50, and one sample (ā titre 45) was negative by the 3T-6 assay.

## DISCUSSION

PV infected mice excrete the virus in copious amounts in their urine, and, to a lesser extent, in saliva and faeces. Being relatively resistant to dehydration, PV can remain viable in bedding for prolonged periods. Other micro-organisms similarly excreted and resistant to dehydration can also survive in bedding. These organisms in the bedding can become airborne when bedding is disturbed by personnel activity and especially during cage changing. A range of 50–200 colony forming units of bacteria was detected during each activity (McGarrity *et al.* 1969).

The majority of these airborne particles are relatively large and sediment within 10–15 min. Using PV as an indicator, studies showed that enough PV remain airborne to infect sentinel mice (McGarrity *et al.* 1976). Even though sentinel mice became infected via the airborne route, circulating antibodies were not detected in any of 138 human sera, including sera from 29 animal care technicians who handled PV infected mice and 15 personnel who worked with the virus (McGarrity *et al.* 1976).

The technique of high volume air sampling and biophysical concentration enabled small amounts of PV to be detected. The air that was sampled in these assays was concentrated a total of $7{\cdot}5 \times 10^7$, apparently without physical damage to the PV as indicated by cell culture assay and MAP. Although PV may be more resistant to environmental stress than many other viruses, the combined use of high volume air sampling and biophysical concentration should be investigated for other viruses. Detection of small amounts of airborne viruses may be significant in epidemiology and biohazard research.

The air sample that yielded a total of $3 \times 10^3$ TCID50 had an airborne PV concentration of $3 \times 10^3$ TCID50/1100 l/min $\times$ 60 min/sample = 0·045 TCID50/l or 1 TCID50 in 22·2 l of air. In previous animal studies, 50 % of susceptibles were infected through the airborne route after approximately 9 h of exposure, i.e. 1 ID50 = 9 h $\times$ 1·44 l of air inhaled/h by each mouse = 13 l (McGarrity *et al.* 1976).

The relative efficiency of the air sampling and cell culture assay described here is approximately 58·6 % that of the animal infection system (13 l/22·2 l). This is a high efficiency, considering the uncertainties and variables of air sampling. Also, animal inoculation is a more sensitive method to detect PV than cell culture (Yabe *et al.* 1961). Nevertheless, the combination of HVAS, appropriate concentra-

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tion procedures and sensitive assay systems can be a valuable epidemiological tool to detect small amounts of airborne virus.

These studies were supported by Grant RR00903 from the Division of Research Resources, NIH. The technical assistance of Victoria Ammen, Charlene Williams and Diane Meredith is gratefully acknowledged. The high volume air sampler was supplied through the courtesy of Dr Emmett Barkley, Office of Research Safety, National Cancer Institute, Bethesda, Md.

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