FIELD STUDIES ON COLIPHAGES AND COLIFORMS AS INDICATORS OF AIRBORNE ANIMAL VIRAL CONTAMINATION FROM WASTEWATER TREATMENT FACILITIES

K. F. FANNIN,* J. J. GANNON, K. W. COCHRAN and
J. C. SPENDLOVE

Department of Environmental and Industrial Health and of Epidemiology, School of Public Health,
The University of Michigan, Ann Arbor, Michigan 48109, and Dugway Proving Ground,
Dugway, Utah 84022, U.S.A.

Abstract—The occurrence of animal viruses in the aerosol emissions of wastewater treatment facilities was evaluated by direct assay and by the use of coliforms and coliphages as indicator organisms. Coliforms and coliphages were compared and evaluated with regard to their suitability as indicators of airborne animal viral contamination from wastewater treatment facilities. Two plants, one with treatment by activated sludge and the other by trickling filtration, were studied. Field air sampling procedures used large-volume air samplers, with recirculation devices, and Andersen samplers. Airborne viruses were enumerated by a most probable number (MPN) procedure. Partially treated liquid sewage contained about 1.0×10^2 pfu 1^{-1} of animal viruses assayed on Buffalo Green Monkey (BGM) cells, 3.6×10^5 and 5.0×10^5 pfu 1^{-1} of coliphages, depending upon the *E. coli* host strain used for assay, and 2.0×10^9 colonies 1^{-1} of coliform bacteria. No airborne animal viruses were recovered, airborne coliphage levels averaged 2.3×10^{-1} and 3.0×10^{-1} MPN m^{-3}, coliforms from aerosol emissions were 2.1×10^2 colonies m^{-3}. Ratios of coliphages to animal viruses indicate that wastewater treatment plants may be continuous sources of low level concentrations of animal virus aerosols. Evidence shows coliforms to be much less stable than coliphages in the airborne state. Coliphages may be a more acceptable indicator of airborne animal viral contamination than coliforms.

INTRODUCTION

Certain bacteria have been shown to be emitted in relatively high concentrations from wastewater treatment facilities (Randall and Ledbetter, 1966). Although animal viruses, many of which are pathogenic, have been shown to occur throughout the processes of wastewater treatment, their airborne emission from these sources has not been evaluated. Since animal viruses are probably present in these airborne emissions in very low concentrations, the use of indicator organisms such as coliforms or coliphages may be suitable for early estimates of the levels.

The use of coliphages as indicators of animal viral water pollution has been advocated by Kott *et al.* (1969). Metcalf *et al.* (1970) pointed out, however, that it may be difficult to establish the validity of coliphages as viral indicators, since they may be present in high numbers even when enteroviruses are in low concentrations or absent. But, since coliphages behave similarly to the enteroviruses and are more resistant to environmental stresses than are the coliforms, it has been argued that they may be used to evaluate more accurately the effectiveness of waste-water treatment than the traditional bacterial indicator organisms (Kott *et al.*, 1969).

Caution must be observed in the use of a nonpathogenic organism as a model for the behavior of a pathogenic one (Metcalf, 1969). But increasing evidence indicates that, under certain conditions, some environmental factors may similarly influence the survival of both bacterial and animal viruses. Kott *et al.* (1969), for example, cited evidence that the T-1 to T-7 coliphage group is as resistant to the natural killing properties of sea water as are the enteroviruses. These findings were supported by Metcalf *et al.* (1970), who found that coliphage was able to survive at least as long as coxsackievirus B-3 in estuaries and in shellfish. Much of the effort to determine the stability of coliphages and to study their suitability as animal virus models has been directed toward the T bacterial viruses. The study of aerosols of these T-phages has shown marked variations in the stability of various airborne agents within this group. Harstad (1965) found that T-1 phage aerosols were more stable than those of T-3 phage. Ehrlich *et al.* (1964) showed that the survival of airborne T-3 phage was similar to Rous-sarcoma virus and poliomyelitis virus, with higher survival rates at higher levels of relative humidity.

Other investigators have maintained, however, that the more recently isolated small RNA phages (Loeb,

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* Present address: Life Sciences Research Division, IIT Research Institute, 10 West 35th Street, Chicago, Illinois 60616, U.S.A.

1959; Davis et al., 1961) may be better models of the enteroviruses than are the T-phages, since they share a greater number of similar characteristics. Olivieri (1974), for example, advocated the use of f_2 phage as an enterovirus model by stressing its similarities with poliovirus and its ease of manipulation. Lefler and Kott (1974) found that f_2 phage survived longer in sand columns than poliovirus did. Kott et al. (1974) used f_2 and MS-2 phages as reference strains for studies on their resistance to environmental stress. They found f_2 to be more resistant to chlorination in oxidation pond effluents than MS-2 phage, which was more resistant than poliovirus type 1. This report did not, however, distinguish between free and combined chlorine. Scarpino (1975) clearly demonstrated poliovirus 1 and coxsackievirus A9 to be more resistant than MS-2 or f_2 (which were more resistant than E. coli or T-5 phage) to inactivation by hypochlorous acid. He further pointed out that f_2 is more resistant than poliovirus 1 or coxsackievirus A9 to inactivation in the presence of monochloramine. Dubovi and Akers (1970) supported the use of tailless bacteriophages such as DNA-containing S-13 and RNA-containing MS-2 instead of the T bacterial viruses which possess a tail-like structure used in attachment to bacteria. They indicated that factors which damage this structure during aerosol studies may eliminate the infectious capacity of the particle. These authors found that, because of similarities in structure and in mechanism of the inactivation in this airborne state, these viruses were very similar to certain animal viruses and justifiable models for certain types of aerosol studies. While the intact MS-2 virus may be inactivated in aerosols, the infectivity of the RNA has been found to remain stable (Dubovi, 1971). This observation is similar to that observed with poliovirus (deJong et al., 1973).

Coliform bacteria have been used as indicators of fecal contamination of water for many years and their densities have been utilized as "criteria of the degree of pollution and thus of the sanitary quality of the sample under examination" (Standard Methods, 1971). Some investigators have suggested that these organisms would serve equally well as indicators of airborne contamination by wastewater treatment plants (Adams and Spendlove, 1970; Goff et al., 1973). Randall and Ledbetter (1966), however, suggested that Klebsiella would be a better indicator of bacterial air pollution from sewage sources, since these encapsulated bacteria are considered to be more capable of survival in the atmosphere than nonencapsulated ones. While such conclusions for Klebsiella may be valid, the procedures for their differentiation are generally more involved than those required for coliform observations. Since coliform organisms are universally accepted as indicators of fecal contamination, they were examined as possible indicators of contamination by airborne viruses from wastewater treatment plants. The purpose of this study is to evaluate animal virus occurrence in aerosol emissions from waste-

water treatment facilities by direct assay and by the use of coliforms and coliphages as indicator organisms and to evaluate and compare the suitability of coliforms and coliphages as animal virus indicators.

MATERIALS AND METHODS

Field sampling

Two wastewater treatment plants were selected for the comparative study of airborne coliphages, coliforms and animal viruses. These plants have been described by Fannin et al. (1976) as plants No. 3, activated sludge, and 4, trickling filter, both in southeastern Michigan. Airborne samples for coliphage and animal virus assay were taken with Large Volume Air Samplers (LVAS) manufactured by Litton Systems, Inc., Minneapolis, Minnesota, recirculating the phosphate sampling fluid (PSF), and processing samples as previously described (Fannin et al., 1976). Coliform bacteria were collected with battery-operated Andersen samplers which were loaded with glass Petri dishes, each containing 27 ml of Endo agar.

Sewage processing

Liquid sewage samples were processed for animal viruses after a procedure described by Rao et al. (1972), with slight modification. A 500 ml sample was blended at high speed in a Waring blender for 4 min, followed by centrifugation at 940 g for 30 min at 4°C, for the removal of gross particulate matter. The pH was adjusted to 3.0 with 0.3 N HCl, and the sample was centrifuged 13,400 g at 4°C for 30 min. AlCl$_3$ was added to the supernatant to a final concentration of 0.005 M, as described by Wallis et al. (1972). The sample was then slowly pressure filtered, using filtered air, through a 47 mm dia. Millipore filter, 0.45 μm pore size. The filtering apparatus, with filter intact, was then transferred to a 50 ml filtering flask. Five ml of 3% beef extract was added to the filter and placed at 4°C for 30 min. Following this period, the remaining elution broth (beef extract solution) was pressure filtered into the flask. Samples for coliphage assay were obtained from the above sample following the 940 g centrifugation step and filtered as described by Fannin et al. (1976). A portion of the sample was also removed following the blending step for subsequent total coliform assay.

Coliform and coliphage assays

Airborne coliphage isolation and enumeration procedures used the most-probable-number (MPN) procedure described by Kott (1966) as modified (Fannin et al., 1976). The MPN method has been used to estimate phage concentrations at levels which were 100- to 1000-fold lower than could be detected by plaquing (Hoff and Jakubowski, 1966). Results are reported as MPN m^{-3}. Concentrations of airborne coliform bacteria containing particles were determined by colony counts of exposed Andersen sampler plates after incubation at 37°C for approximately 24 h. All counts were corrected for positive hole counts as reported by Andersen (1958). Results are reported as colonies m^{-3}. Liquid-borne coliphages were assayed by the soft agar-overlay method described by Adams (1959), using 2 replicates for each dilution. Plates were incubated for 5–18 h, plaques were then counted and reported as plaque-forming units (pfu) per liter. Total coliform assay was performed according to Standard Methods for the Examination of Water and Wastewater (1971) by the membrane filter procedure, using 2 replicates at each dilution.

Animal virus assays

Since very low concentrations were anticipated, the MPN method was also used for the enumeration of air-

borne animal viruses. Chang (1965) has discussed evidence that the MPN method is more sensitive to very low animal virus levels than is the plaque method. Animal viruses were assayed in cultures of Buffalo Green Monkey (BGM) cells using 10 tubes per processed airborne sample. Each tube was washed three times with the following solution: Hanks Balanced Salt Solution (HBSS), containing 200 units ml^{-1} penicillin, 200 $\mu g\ ml^{-1}$ streptomycin, 1 $\mu g\ ml^{-1}$ Fungizone, pH adjusted to 7.0–7.2 with 7.5% sodium bicarbonate solution. After washing, 0.5 ml of processed airborne sample was added to each tube. Control groups of 10 tubes each were inoculated; 0.5 ml of HBSS or PSF were included. One milliliter of Minimum Essential Medium (MEM) containing 2% inactivated fetal calf serum was added to each tube. Tubes were capped, incubated at 37°C for 2 days and then checked microscopically for cytopathic effect (CPE). Each tube which showed CPE was subinoculated into other culture tubes three times for confirmation of positive virus isolation.

Liquid sewage samples were assayed for animal virus by plaquing on BGM cells in 2-oz prescription bottles. Cell monolayers were washed three times as described above. Control bottles inoculated with HBSS or 3% beef extract in HBSS were included in each test. Five-tenths milliliter of each sample-eluate or control was added to each of 10 culture bottles and spread evenly. After incubation for 1.5 h to permit virus absorption, cells were overlaid with 5 ml of MEM plaquing medium, without neutral red. The MEM plaquing medium consisted of MEM in HBSS with 1.5% Bacto Agar, 1% of a 50% aqueous solution of $MgCl_2 \cdot 6H_2O$, 2% heat-inactivated fetal calf serum, 200 units m^{-1} penicillin, 200 $\mu g\ ml^{-1}$ streptomycin, 1 $\mu g\ ml^{-1}$ Fungizone; pH adjusted to 7.0–7.2 with 7.5% solution of $NaHCO_3$. The cultures were inverted, incubated at 37°C for 2 days, at which time they were overlaid with 4 ml of MEM plaquing medium containing 4% 1:1000 neutral red, and incubated in the dark for 1 more day. Plaques were marked, counted and cultures incubated for 4 more days, with plaques being marked and counted at daily intervals. Virus concentration was expressed as plaque-forming units (pfu) per liter of liquid sewage.

Efficiency of recovery of animal virus from wastewater

Sewage influent was obtained from the influent trickling filter boom at wastewater treatment plant No. 4. This wastewater was then diluted into aliquots and autoclaved. A polio type 1 LSc2ab suspension was added to autoclaved wastewater. This sample was then processed according to the modified membrane filter concentration procedure as described above. Two-fold dilutions of the eluate in HBSS were assayed on BGM cells by the plaquing procedure described above. Eluate controls were included as 2-fold dilutions of 3% beef extract. Virus controls of an aliquot

Table 2. Efficiency of recovery of Polio 1 LSc2ab from sewage by membrane filter concentration procedure

Sewage volume (ml)	Virus input (pfu)	Virus recovery (pfu)	Recovery (%)
240	1000	462	46
240	1000	352	35
230	390	228	58
208	353	150	43

Mean = 46%.

of the original virus input were simultaneously assayed at 10-fold dilutions in HBSS. Plaques were counted and expressed as pfus. Percentage of recovery was determined by comparing the pfus recovered to actual pfu input.

RESULTS

Average animal virus levels recovered from partially treated sewage, taken from trickling filter booms and activated sludge units of wastewater treatment plants, were 1.0×10^2 pfu l^{-1} (see Table 1). The efficiency of the animal virus concentration procedure used in this study averaged 46% of the total input of polio 1 LSc2ab from autoclaved sewage (see Table 2). Average coliphage levels in this sewage were 5.0×10^5 pfu l^{-1} for phages of E. coli C3000 and 3.6×10^5 pfu l^{-1} for phages of E. coli K12HfrD (see Table 1). As shown in Table 3, coliform organisms averaged 2.0×10^9 colonies l^{-1} in these liquid sewage samples.

No airborne animal viruses were recovered from the environments studied. Average airborne coliphage

Table 3. Airborne coliforms related to liquid sewage concentration

Plant No.	Liquid sewage concn. (colonies/l)	No. of air samples	Mean airborne concn. (colonies/m³)
3	3.2×10^8	3	1.3×10^2
3	3.2×10^8	1	1.8×10^2
3	7.8×10^9	4	3.9×10^2
4	1.5×10^9	4	7.9×10^1
4	5.5×10^7	4	2.1×10^1
Mean	2.0×10^9		2.1×10^2

Table 1. Airborne viruses related to liquid sewage concentration

Plant No.	Concentration of viruses assayed on:						No. of air samples
	BGM Cells		E. coli C3000*		E. coli K12HfrD*		
	Sewage (pfu/l)	Air (MPN/m³)	Sewage (pfu/l)	Air (MPN/m³)	Sewage (pfu/l)	Air (MPN/m³)	
3	7.0×10^1	0	5.9×10^4	2.6×10^{-1}	2.1×10^5	1.7×10^{-2}	3
3	5.6×10^1	0	3.9×10^4	1.1×10^{-1}	1.5×10^5	0	1
3	ND†	0	7.4×10^4	$\geq 5.1 \times 10^{-1}$	4.2×10^5	3.9×10^{-1}	4
4	1.1×10^2	0	8.1×10^5	$\geq 2.9 \times 10^{-1}$	5.8×10^5	$\geq 3.8 \times 10^{-1}$	4
4	1.8×10^2	0	8.8×10^5	1.9×10^{-1}	4.6×10^5	1.4×10^{-1}	4
Mean	1.0×10^2	0	5.1×10^5	3.0×10^{-1}	3.6×10^5	2.3×10^{-1}	

* These data are taken in part from Fannin et al. (1976).
† Sample not assayed.

Table 4. Comparison of ratios of Coliforms to coliphages from airborne emissions and liquid sewage

Plant No.	No. of samples	Coliform (a)	E. coli C3000 phage (b)	(a/b)	E. coli K12HfrD phage (c)	(a/c)
		Airborne emission $\overline{X}/m^3$	$\overline{X}/m^3$		$\overline{X}/m^3$	
3	8	2.7×10^2	3.7×10^{-1}	7.3×10^2	2.0×10^{-1}	1.4×10^3
4	8	5.0×10^1	2.4×10^{-1}	2.1×10^2	2.6×10^{-1}	1.9×10^2
		Liquid sewage $\overline{X}/1$	$\overline{X}/1$		$\overline{X}/1$	
3	3	2.8×10^9	2.8×10^5	1.0×10^4	3.6×10^5	7.8×10^3
4	2	7.8×10^8	8.4×10^5	9.2×10^2	5.2×10^5	1.5×10^3

Table 5. Comparison of coliform/coliphage ratios of liquid sewage to airborne emissions

Plant No.	E. coli C3000* Liquid sewage ratio (a)	Airborne ratio (b)	(a/b)	E. coli K12HfrD* Liquid sewage ratio (c)	Airborne ratio (d)	(c/d)
3	1.0×10^4	7.3×10^2	13.7	7.8×10^3	1.4×10^3	5.8
4	9.2×10^2	2.1×10^2	4.4	1.5×10^3	1.9×10^2	7.8

* Ratio includes coliphages as determined by this host.

Table 6. Airborne emissions of coliforms and coliphages compared to windspeed

Wind velocity (mph)	No. of samples	Mean concn/m³ of air Coliforms	E. coli C3000 Phage*	E. coli K12HfrD Phage*
<1.0	9	6.2×10^1	2.5×10^{-1}	2.3×10^{-1}
1.0–2.9	5	2.7×10^2	3.3×10^{-1}	2.3×10^{-1}
3.0–5.9	2	3.2×10^2	4.6×10^{-1}	2.0×10^{-1}

$r_s = 0.67$ $r_s = 0.41$ $r_s = 0.09$
$t_{(n-2)} = 3.38*$ $t_{(n-2)} = 1.68$ $t_{(n-2)} = 0.34$
significant   not significant   not significant

* These data are taken in part from Fannin et al. (1976).

levels were 3.0×10^{-1} MPN m^{-3} for E. coli C3000 phages and 2.3×10^{-1} MPN m^{-3} for E. coli K12HfrD phages. Average airborne coliform levels were 2.1×10^2 colonies m^{-3}.

The mean ratio of airborne isolates to liquid sewage concentration was 6.0×10^{-7} for E. coli C3000 phage and 6.7×10^{-7} for E. coli K12HfrD phage. Comparing these ratios to animal virus liquid sewage levels, when isolations were made, the expected airborne concentration of animal viruses would be approximately 6.2×10^{-5} or 6.5×10^{-5} MPN m^{-3}, assuming that these viruses behave similarly to coliphages under the conditions studied. The mean airborne-to-liquid sewage coliform ratio was 1.1×10^{-7}, which is about 0.8 log lower than that observed with the bacterial viruses. Comparing this ratio to animal virus liquid sewage levels, the expected airborne animal virus concentrations would be approximately 1.1×10^{-5} MPN m^{-3}. Thus, the estimates of the con-

centration of airborne animal virus, using a coliform bacteria ratio, would be about 0.8 log lower than those made with coliphage ratios.

An observation of the ratio of coliform bacteria to E. coli phage airborne isolates from plants Nos. 3 and 4 is shown in Table 4. This ratio ranges from 1.9×10^2 at plant No. 4 to 1.4×10^3 at plant No. 3. Applying the Mann–Whitney U-test, as described by Siegel (1956), these data show that the probability of these ratios coming from the same population is 0.013 for those involving E. coli C3000 phage and 0.001 for those obtained with E. coli K12HfrD phage. That is, there is a significant difference between the ratio from these plants.*

This observation is examined further in Table 4, where the ratio of coliform bacteria to coliphage in liquid sewage also appears greater in plant No. 3 than in plant No. 4 for the coliphages of both hosts examined. Comparing the liquid sewage ratios observed in Table 4 to the airborne ratios, Table 5 illustrates that, based upon these observations, the coliform-to-coliphage ratio in liquid sewage is consistently higher

* 95% confidence levels are used for determination of significance of all statistical tests in this investigation.

Table 7. Airborne emissions of coliforms and coliphages compared to ambient air temperature

Temp. (°C)	No. of samples	Mean concn/m³ of air		
		Coliforms	E. coli C3000 Phage*	E. coli K12HfrD Phage*
15.0–19.9	8	2.5×10^2	3.5×10^{-1}	2.3×10^{-1}
20.0–24.9	6	6.6×10^1	3.0×10^{-1}	2.1×10^{-1}
25.0–29.9	2	8.3×10^1	1.0×10^{-1}	2.8×10^{-1}

$r_s = -0.62$ $r_s = -0.22$ $r_s = -0.15$
$t_{(n-2)} = 2.96*$ $t_{(n-2)} = 0.84$ $t_{(n-2)} = 0.57$
significant   not significant   not significant
* These data are taken in part from Fannin et al. (1976).

Table 8. Airborne emissions of coliforms and coliphages compared to relative humidity

Relative humidity (%)	No. of samples	Mean concn/m³ of air		
		Coliforms	E. coli C3000 Phage*	E. coli K12HfrD Phage*
25–39	3	1.1×10^2	1.8×10^{-1}	0.0
40–54	1	1.6×10^2	3.5×10^{-1}	5.0×10^{-2}
55–69	3	7.5×10^1	3.2×10^{-1}	1.7×10^{-1}
70–84	7	2.3×10^2	2.9×10^{-1}	3.1×10^{-1}
85–99	2	7.5×10^1	4.8×10^{-1}	4.8×10^{-1}

$r_s = 0.10$ $r_s = 0.34$ $r_s = 0.70$
$t_{(n-2)} = -0.38$ $t_{(n-2)} = 1.35$ $t_{(n-2)} = 3.67$
not significant   not significant   significant
* These data are taken in part from Fannin et al. (1976).

Table 9. Coliphage and coliform concentrations related to distance from airborne emission source

Organism	Plant No.	Distance from source				U value
		≤ 5 M		> 5 M		
		No. of samples	Mean concn/m³	No. of samples	Mean concn/m³	
E. coli C3000 phage	3,4	12	3.2×10^{-1}	4	2.4×10^{-1}	17.50 (not significant)
E. coli K12HfrD phage	3,4	12	2.5×10^{-1}	4	1.6×10^{-1}	21.00 (not significant)
Coliform bacteria	3,4	12	2.1×10^2	4	1.4×10^1	2.50 (significant)

than the same ratio in airborne emissions recovered from this source. That is, there appear to be greater numbers of coliform bacteria per coliphage in the liquid sewage than after droplets from this liquid become aerosolized.

A strong positive correlation, determined by the Spearman rank correlation coefficient (r_s), as described by Siegel (1956), between windspeed and airborne coliform concentrations, is shown in Table 6. However, no significant correlation is observed with the airborne coliphages of either strain of E. coli studied and windspeed. A significant inverse correlation is shown in Table 7 between airborne coliform concentration and ambient air temperature. That is, as

the temperature increased, the aerosolized coliform recoveries decreased. No significant correlation could be detected between airborne coliphage concentrations and ambient air temperature.

Although it is difficult to separate the influence of relative humidity (RH) from other meteorological parameters, it is noted in Table 8 that a stronger positive correlation is observed between RH and airborne coliphage recoveries than between this parameter and aerosolized coliform levels. A significant correlation is found with the phages of E. coli K12HfrD and RH.

Significantly lower coliform levels were observed at sampling distances greater than 5 m from the source

than at nearer locations (see Table 9). No significant difference could, however, be detected between coliphage recoveries from the two general locations. That is, coliform recoveries significantly decreased at points farther from the emission source while coliphage levels remained more constant.

DISCUSSION

When comparing the levels of coliphages to animal virus in liquid sewage, it is not surprising that no airborne animal virus isolations were made from aerosols. The mean animal virus concentration per liter was about 3.6–3.7 logs lower than the mean coliphage level. On comparison of ratios of mean airborne coliphage isolations and their liquid sewage concentrations to animal virus liquid sewage levels, the expected airborne animal virus concentration would be approximately 6.2×10^{-5} or 6.5×10^{-5} MPN m^{-3}, depending upon the phage host used for the estimate and assuming that these agents behave similarly in the environments studied. In other words, approximately 15×10^3–16×10^3 m^3 of air would need to be sampled to provide 1 detectable animal virus tissue culture unit under the conditions of this study. Based upon these estimates, the needed volume of air to be sampled and assayed to attain animal virus isolations within the range of those observed for coliphages would be at least 3.5×10^3 m^3. In this study, the maximum volume of air sampled during any particular sampling run was 1.20×10^2 m^3, and the sampled fluid available for animal virus assay was one-third or less of the total amount collected, since the remaining two-thirds was allocated to coliphage assay. Consequently, the level of sensitivity of the procedures used appears to be below that required for airborne animal virus isolations, under the conditions of sample collection and assuming that these viruses behave similarly to the coliphages isolated on specific host cells.

Wastewater treatment plants are continuous sources of airborne emissions to which downwind populations may be exposed. The significance of this exposure is related to the physical and biological stability in air of the potentially pathogenic emitted material and to the susceptibility, size, and density of an exposed downwind population. As this population increases in size and density, the probability of infection with airborne agents also increases. The minimal estimated levels of animal viruses in the airborne emissions studied in this investigation would produce considerable public health concern if they are shown to reach large susceptible downwind populations.

Airborne coliform/coliphage ratios were lower than liquid sewage coliform/coliphage ratios (see Table 5) at both plants examined. In other words, a higher number of coliforms per coliphage was recovered from the liquid sewage than from the air. Assuming both types of agents had the same opportunity for aerosolization and recovery, this observation indicates that the survival of airborne coliforms was lower than the survival of airborne coliphages.

Dispersion of airborne particles to sites remote from the source is influenced greatly by atmospheric stability. Stability of the atmosphere is highly dependent upon the vertical temperature gradient and the height of its distribution. Although rough estimates of stability may be made by visually observing certain climatic conditions, no attempt was made to include such estimates in this study. Since atmospheric stability could neither be controlled or measured, the results of this study must be interpreted with this in mind. Under natural conditions, such as those which exist in the field environment, it is difficult to separate the effects of individual meteorological parameters on the survival of airborne microorganisms. Although the conditions which existed during this investigation varied widely, attempts were made to determine whether correlation was observed between the recovery of certain airborne microorganisms and some meteorological parameters.

The present study showed that airborne coliform recoveries were significantly reduced under conditions of lower wind velocity (see Table 6), higher ambient air temperature (see Table 7), and at increased distances from the emission source (see Table 9). Coliphage recoveries were not found to be as strongly influenced by these conditions. Increases in wind velocity reduced the amount of time for which coliform-containing particles remained airborne prior to collection. This condition resulted in decreased exposure of the coliform organisms to the stresses of the airborne state and, hence, may have enhanced their chances of being recovered at the sampling stations. Wind velocity may have been more critical to coliforms than to coliphages, since the former agents appear to be much less stable in aerosols. The negative effect on the survival of airborne coliforms with increases in ambient temperature from these plants agrees with the findings of Poon (1966) who showed that, under laboratory conditions, E. coli viability decreased logarithmically as temperature increased. He also demonstrated that this lower survival rate occurred at higher temperatures over a wide range of RH levels. Lower coliform concentrations at distances farther from this source were also shown by other investigators (Goff et al., 1973; Randall and Ledbetter, 1966). The lesser decrease for coliphages may be explained by the longer survival of coliphage agents compared to coliform bacteria in an aerosolized state. However, significant positive correlations were observed between the recoveries of coliphages of E. coli K12frD and increases in RH. Lower correlation was found between RH and E. coli C3000 phages, and no correlation could be made between coliform recovery and RH levels.

Although these observations may have been affected by additional environmental factors, they are consistent with the findings of other investigators who showed that coliphages are generally more stable at

higher RH levels (Hatch and Warren, 1969; Dubovi and Akers, 1970; Benbough, 1971), and that no linear correlation exists between *E. coli* survival and RH (Webb, 1959).

The stability of organisms in the coliform group appears to be lower than that observed for certain viruses. Studies on the airborne stability of MS-2 by Dubovi and Akers (1970) showed, for example, that the survival of these agents was similar to that of polioviruses. Scarpino (1975) showed that poliovirus 1, coxsackievirus A9, MS2, and f$_2$ were much more resistant than coliforms to the extreme environmental influences of contact with hypochlorous acid than coliform bacteria. Geldreich and Clarke (1971) also cited evidence that certain enteric viruses are more resistant to chlorination than are coliform bacteria.

This evidence indicates that, under extreme conditions such as aerosolization or chlorination, both animal and bacterial viruses may persist longer as viable agents than coliform bacteria under similar conditions. It is desirable to attain an estimate of animal virus concentrations by direct assessment of their levels. The detection of coliforms or coliphages is, however, easier, faster, more economical, and probably more accurate than present methods of detecting low levels of animal viruses from the environment.

Based upon the above observations, the use of coliform bacteria as indicators of the viral contamination of these environments may result in an inaccurate assessment of the true presence or survival of viruses. Coliform determinations may not, therefore, be a valid or acceptable indicator of viral contamination.

No conclusive coliphage-to-animal virus ratio can be established from the present investigation. A pattern of *Escherichia coli* B coliphage–enteric virus ratio could not be established by Moore *et al.* (1974) during the monitoring of raw wastewater entering a Texas wastewater treatment plant. It was indicated by Vaughn and Metcalf (1975) that coliphages may be able to replicate in the sewage under certain conditions, thus upsetting any original ratio which might have existed with animal viruses. It is also likely, however, that the conditions which would permit coliphage replication in the environment might also permit coliform organisms to reproduce themselves, thus making these bacteria much less reliable indicator organisms.

It is, on the other hand, very unlikely that the conditions permitting significant replication of coliforms or of coliphages would exist in the airborne state. The air is a hostile environment to these organisms and survival depends upon a large number of factors, many of which have been discussed. The recovery of coliforms and coliphages downwind from a source indicates that they have been dispersed to that location and have survived aerosolization. Since certain coliphages were shown to survive this environment better than coliform bacteria, they should serve as better estimates of the dispersion of viable virus particles emitted from the source. Based upon evidence that certain coliphages, such as MS-2, are at least as hardy as certain animal viruses, such as polio, and that their airborne behavior is similar, certain bacterial viruses would serve as a better airborne viral indicator than coliforms. Determination of coliphage densities in a source and their subsequent airborne concentrations at an adjacent or downwind site, while establishing the animal virus levels of that source, would permit one to estimate the airborne animal virus levels which might exist, assuming similar survival patterns and without the use of more elaborate air sampling equipment.

CONCLUSIONS

1. Coliphage, animal virus ratios between liquid and aerosolized sewage indicate that wastewater treatment plants may be continuous sources of low level concentrations of animal virus aerosols.

2. Coliforms are an unreliable indicator of airborne viral contamination.

3. Coliphages are a more acceptable indicator of airborne viral contamination than coliforms in a sewage treatment environment.

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