INITIAL SURVEY FOR VIRUSES IN LAKE GEORGE

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A preliminary investigation to determine the presence of viruses or virus like agents was conducted in Lake George, New York. Although some indication was given regarding the presence of certain of these agents within this freshwater lake, little evidence concerning the full spectrum of virus type(s) or their concentration was offered. This reconnaissance study attempted to confirm previous suspicions that virus agents were present in Lake George and hoped to offer preliminary identification of any suspect particles.

Water samples (2-5 liters) were secured via the grab technique from the surface of West Brook which enters Lake George at the southern end of the lake on May 24, 1972. This locale was chosen as a sampling site since this stream is suspected of receiving wastewater effluent from the municipal treatment plant at Lake George Village prior to its discharge into the lake itself. Samples taken on November 30, 1971 in a similar fashion from Dunham Bay and Echo Bay were also examined. In all cases the samples were concentrated to screen for the presence of viruses using the double phase polymer method of Albertsson (1) with some minor modifications. These modifications have been examined previously and did not alter the efficacy of this method in any fashion (3). The complete methodology has been described (1, 3).

HeLa cells were cultivated in Eagle's MEM with Earle's salts in an atmosphere comprised of 5% CO_2- 95% O_2 in a water jacketed CO_2 incubator (National) at 38°C according to standard methods (5). In determining the presence and number of animal viruses, HeLa cell cultures were drained of their growth medium and fresh medium, lacking in calf serum and containing the suspected virus sample, was added to the cell sheet and incubated at 38°C for one hour. Animal virus plaque assays were done according to the classic method of Dulbecco and Vogt (2).

The preparation of Escherichia coli B as host bacterium in all coliphage infectivity studies was done as follows. Either a loop-ful of stock E. coli B from an agar slant or 0.3 ml from a stock culture of this bacterium in MS Broth (MSB) was aseptically transferred to 30 ml sterile MSB and aerated via shaking at 37°C at 180-200 rpm for 18 to 24 hours. One-tenth ml (ca. 5×10^4 cells/ml) from this culture per 10 ml fresh MSB was then incubated at 37°C under the same conditions for $2\frac{1}{2}$-3 hours (Cell concentration = ca. 5×10^8 cells/ml). The concentrated water sample(s) was then assayed using this bacterial suspension as host organism according to Hershey et al. (4).

Table 1 indicates that a reasonable amount of coliphage exists in the samples obtained from West Brook ($2.1-2.25 \times 10^3$ phage/ml) to warrant additional investigations. There was, however, no evidence of coliphage in those samples examined from both Echo Bay and

Dunham Bay.

TABLE 1: COLIPHAGE ASSAY FROM LAKE GEORGE [a]

SAMPLING LOCALE	DILUTION AND PLAQUE NUMBER [b]					
	U	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Echo Bay	-	-	-	-	-	-
West Brook	225	21	-	-	-	-
Dunham Bay	-	-	-	-	-	-

a. 0.1 ml sample employed throughout the assay

b. Titer (S) as Plaque Forming Units per ml (PFU/ml) are:

Echo Bay = -

West Brook= $2.1 - 2.25 \times 10^3$

Dunham Bay= -

The time of sampling for Echo Bay and Dunham Bay (November 30, 1971) may be a critical factor in explaining the absence of these particles since the potential viral load from sewage in Lake George at this time is probably minimal due to the decreased concentration of people at the lake during this season. Thus, the need to determine the presence of viruses not only in inlet streams (as shown for West Brook) but also in the open water areas would greatly aid these investigations in determining the persistence of these particles in oligotrophic waters.

The possible presence of animal viruses in Lake George was also examined from those samples obtained and described above. The below data (Table 2) show that the samples tested from West Brook

were capable of eliciting a characteristic plaque response on HeLa cell cultures. However, no plaques resulted from the concentrated

TABLE 2: ANIMAL VIRUS ASSAY FROM LAKE GEORGE[a]

SAMPLING LOCALE	DILUTION AND PLAQUE NUMBER[b]					
	U	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Echo Bay	-	-	-	-	-	-
West Brook	3	-	-	-	-	-
Dunham Bay	-	-	-	-	-	-

a. 0.1 ml sample employed throught the assay

b. Titer(s) expressed as PFU/ml are:

Echo Bay = -

West Brook= 30

Dunham Bay= -

samples examined from both Echo and Dunham Bays. These data are similar to that shown previously for coliphage and may have some interesting implications. Although it is not possible to selectively identify these animal viruses at this time, it is likely that they can be grouped into the enterovirus category. Numerous viruses of human and other animal origin are excreted daily, principally in feces but also via oral secretions; these particles can reach and possibly contaminate water supplies. It is unknown what happens to these multitudes of viruses. The rhinoviruses,for example, may not persist for any lengthy time period and thus are probably not transmitted via water routes since these viruses are unable to

withstand pH changes, temperature alterations or fluctuations and the lack of protective covering offered by feces and other organic materials. These factors do not, however, affect other enteroviruses (viz. polioviruses, ECHOviruses, coxsackieviruses) which are stable and persist in water for long periods. Thus, it appears likely that viruses from human fecal and bacterial sources can find their way into a freshwater body such as Lake George. Conclusive identification of these animal viral agents is not feasible at this time with the available data.

It appears unlikely that a secondary wastewater treatment facility such as that in operation for Lake George Village is capable of removing virus and/ or virus like agents prior to final discharge. In fact, Clarke and Kabler found that certain enteroviruses are not only resistant to bactericidal concentrations of chlorine (3a, 3b, 6) but also emphasized that secondary treatment with trickling filters removes only about 40% of the enteroviruses (3b). Since the viral reduction and/ or elimination efficiency of these trickling filters is questionable and from the data shown in Tables 1 and 2, it appears that further and more comprehensive studies in this area for Lake George are necessary.

The isolation of viruses from freshwaters is obviously important from several viewpoints. Classically, biological analysis of water relies principally on the presence of coliform bacteria (viz. Escherichia coli) as a measure of recent sewage contamination.

However, the absence of this bacterium could indicate the presence of bacterial viruses and thus the presence of undetectable sewage. More data are required, however, for populated areas which employ septic tank-soil absorption systems as their wastewater treatment mechanism prior to discharge into the soil or lake, This and other investigations are to be conducted in the near future.

The investigator wishes to extend his sincere appreciation to the Rensselaer Research Grants Committee for their generosity in allocating these funds for this project. The monies have allowed these preliminary investigations to commence and the data will serve as a basis for future requests to federal and other funding agencies.

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