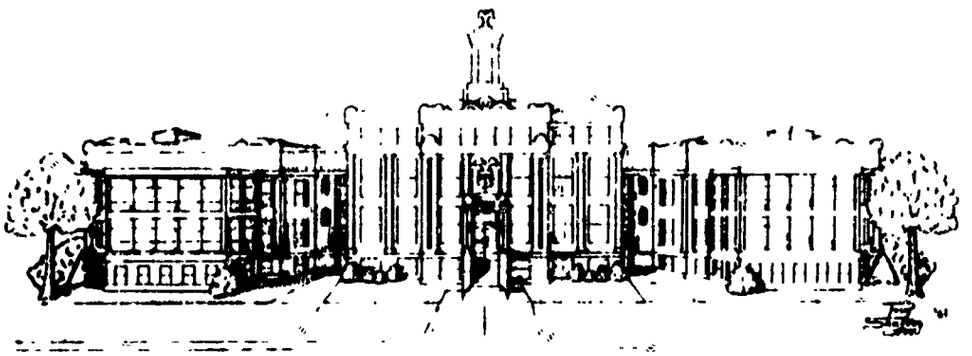
AD 650937

NAVAL MEDICAL RESEARCH

UNIT No. 4



EFFECT OF SEWAGE TREATMENT ON RECOVERY OF POLIOVIRUS FOLLOWING MASS ORAL IMMUNIZATION

E. P. Theios, J. G. Morris, M. J. Rosenbaum

and

A. G. Baker

DEC 3 1967

MF 022.03.07-4016
RU 67.7

FEBRUARY 1967

ARCHIVE COPY

Reprinted from AMERICAN JOURNAL OF PUBLIC HEALTH, Vol. 57, No. 2, February, 1967
Copyright by the American Public Health Association, Inc., 1740 Broadway, New York, N. Y. 10019

The occasion of a mass immunization campaign against polio using trivalent oral poliovirus vaccine was used to study the effect of different sewage treatment methods on the recovery of poliovirus from sewage effluent. Activated sludge treatment process was found far superior to trickling filtration for removal of virus. Chlorination did not eliminate virus recovery. Excretion of virus continued for at least 56 days after vaccination.

EFFECT OF SEWAGE TREATMENT ON RECOVERY OF POLIOVIRUS FOLLOWING MASS ORAL IMMUNIZATION

Eugene P. Theios, M.P.H.; John G. Morris, M.S.C.E.; Max J. Rosenbaum, Ph.D.; and Arthur G. Paker, M.D., M.P.H., F.A.P.H.A.

AN opportunity to compare the effect of two different sewage treatment methods on the recovery of poliovirus from treated sewage effluent was presented during a mass immunization program using trivalent oral poliovirus vaccine in Lake County, Illinois. The use of this immunization program provided a model situation simulating the sewage conditions which may exist during an actual epidemic of these virus types.

The virus content of sewage has been the subject of various reports and several of these investigations have indicated that the activated sludge sewage treatment process is more effective in virus removal than the trickling filtration process.[1-8] An opportunity to confirm these observations could be made by the selection of two communities utilizing these two sewage treatment procedures, respectively. Moreover, the effect of chlorination on viral content of treated effluent before release could also be evaluated.

The extent of excretion of vaccine virus also could be ascertained by obtaining serial samples of sewage after immunization. Since a booster feeding of vaccine was made during the following winter, which is normally regarded as a period of low poliovirus detection, some estimates of survival of virus during this season could also be made.

Materials and Methods

Trivalent oral poliovirus vaccine* was administered to residents of Lake County, Illinois, on a voluntary basis on October 13, 1963. Among the communities participating, Community "A," with a population of 2,400, of whom 1,300 (54.2 per cent) were immunized, and Community "D," with a population of 14,300, of whom 8,000 (56.0 per cent) were immunized, were selected for this study. Community "A" is served by an activated sludge treatment plant with

* Lederle Laboratories, Orimune.

Table 1—Summary of experimental conditions

	Community "A"	Community "D"
Population	2,400	14,300
Number immunized	1,300	8,000
Per cent population vaccinated	54.0%	56.0%
Treatment process at plant	Activated sludge	Trickling filter
Daily flow	241,000 GPD	1,000,000 GPD
Retention time (total)	15.0 hr	6.3 hr
Discharge temperature (avg)	58°F	58°F
Chlorination:		
concentration	2 ppm	0
contact period	45 min	0
Phase I		
Total precipitation* (October 13-November 2)	0.4 in.	
Temperature*: mean	60.5°F	
mean range	52.8–64.3°F	
Phase II		
Total precipitation* (December 8-17)	0.66 in.	
Temperature*: mean	13.3°F	
mean range	3.4–23.1°F	

* Data from US Weather Bureau, O'Hare Field, Chicago, Ill.

final effluent chlorination whereas Community "D" utilizes the trickling filter process (Table 1). The differences in volume of sewage treated approximate the differential in population numbers. In both communities industrial wastes comprised less than 10 per cent of the daily flow.

Investigation of the socioeconomic status of the two populations revealed similar profiles as residential communities although there were differences in population size. A diagrammatic description of the respective treatment plants and sampling points (S.P.) is shown in Figure 1.

The method of obtaining samples for virus study was similar to the gauze pad technic described by MacCallum et al.[9] A five-pound lead weight was wired to one end of the gauze pad and a length of wire to the other. The pad and weight were then lowered into the sewage flow at specific points, the other end wired securely to a support and left for three or four days. Sampling points were the influent and final effluent of each plant and, in the case of the activated sludge process plant, before the chlorination contact chamber.

Samples were collected before mass immunization and then at three- and four-day intervals for 21 days with a follow-up specimen collected 56 days after immunization.

A booster immunizing dose was provided on December 8, 1963, with similar numbers of residents of the communities under study participating. Samples were again collected prior to administration of booster vaccine and at three- and four-day intervals for a ten-day period. In this sample period specimens were also collected from the receiving stream one-half mile below the discharge from the trickling filtration plant on the sixth and tenth day after immunization.

Upon collection, pads were placed in

Table 1—Summary of experimental conditions

	Community "A"	Community "D"
Population	2,400	14,300
Number immunized	1,300	8,000
Per cent population vaccinated	54.0%	56.0%
Treatment process at plant	Activated sludge	Trickling filter
Daily flow	241,000 GPD	1,000,000 GPD
Retention time (total)	15.0 hr	6.3 hr
Discharge temperature (avg)	58°F	58°F
Chlorination:		
concentration	2 ppm	0
contact period	45 min	0
Phase I		
Total precipitation* (October 13-November 2)	0.4 in.	
Temperature*: mean	60.5°F	
mean range	52.8–64.3°F	
Phase II		
Total precipitation* (December 8-17)	0.66 in.	
Temperature*: mean	13.3°F	
mean range	3.4–23.1°F	

* Data from US Weather Bureau, O'Hare Field, Chicago, Ill.

final effluent chlorination whereas Community "D" utilizes the trickling filter process (Table 1). The differences in volume of sewage treated approximate the differential in population numbers. In both communities industrial wastes comprised less than 10 per cent of the daily flow.

Investigation of the socioeconomic status of the two populations revealed similar profiles as residential communities although there were differences in population size. A diagrammatic description of the respective treatment plants and sampling points (S.P.) is shown in Figure 1.

The method of obtaining samples for virus study was similar to the gauze pad technic described by MacCallum et al.[9] A five-pound lead weight was wired to one end of the gauze pad and a length of wire to the other. The pad and weight were then lowered into the sewage flow at specific points, the other end wired securely to a support and left for three or four days. Sampling points were the influent and final effluent of each plant and, in the case of the activated sludge process plant, before the chlorination contact chamber.

Samples were collected before mass immunization and then at three- and four-day intervals for 21 days with a follow-up specimen collected 56 days after immunization.

A booster immunizing dose was provided on December 8, 1963, with similar numbers of residents of the communities under study participating. Samples were again collected prior to administration of booster vaccine and at three- and four-day intervals for a ten-day period. In this sample period specimens were also collected from the receiving stream one-half mile below the discharge from the trickling filtration plant on the sixth and tenth day after immunization.

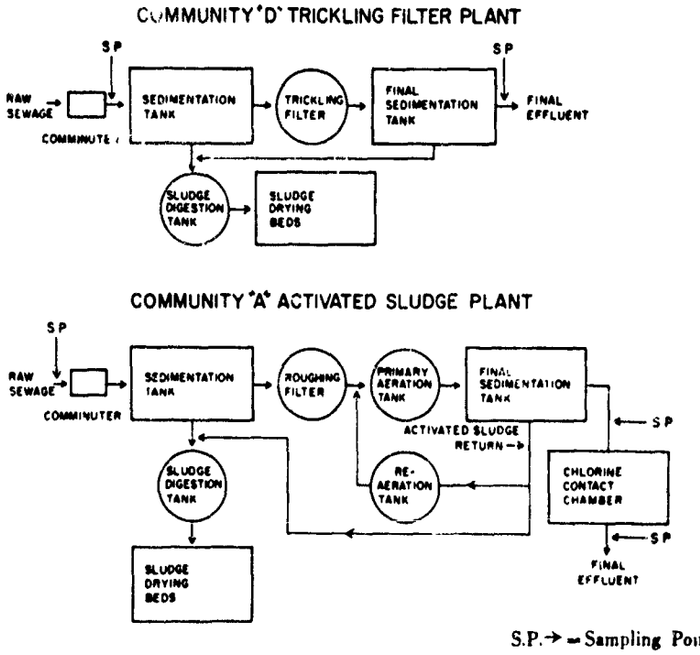
Upon collection, pads were placed in

plastic bags and inserted into wide-mouthed freezer jars and delivered within one and one-half hours to the laboratory. The samples were stored at −20° C until processed for tissue culture inoculation. At that time the pads were thawed and approximately 100 ml of fluid expressed from each. The fluids were centrifuged at 1,000 rpm for ten minutes and 2 ml of supernatant was diluted with 18 ml of Friedman's maintenance media (GYL)[10] and inoculated into monolayers of primary monkey kidney tissue culture prepared in 32-oz bottles. The admixture was allowed to adsorb to the tissue culture for two hours and then decanted. All sheets then were washed and drained with Hank's balanced salt solution and then overlayed with 40 ml of media 199. These bottles were incubated at 36° C and observed daily for cytopathic evidence. Parallel bottle cultures of H.Ep-2 cell monolayers were similarly prepared. When cytopathic effect involved the entire monolayer, the cultures were quick frozen and stored at −70° C for virus identification by neutralization test with type specific poliovirus antisera.

Results

The results of poliovirus isolation attempts are shown in Table 2. It can be observed that no viruses were recovered from the sewage of either community prior to the initial mass immunization. After immunization, varying combinations of the three types of poliovirus were isolated from the influents of both treatment plants for at least as long as 56 days.

Figure 1—Sewage treatment facilities of two Illinois communities, 1963. (Official U. S. Navy photograph)



S.P.→ = Sampling Point

Table 2—Effect of sewage treatment on recovery of poliovirus using both rhesus monkey kidney and H.Ep-2 culture systems

Sample source	Sampling day relative to immunization day (initial vaccination October 13, 1963)							Booster vaccination December 6, 1963			
	(−4)	0† (3)	(6)	(10)	(13)	(17)	(20)	(−1) (56)	0‡ (3) (59)	(6) (62)	(10) (66)
Activated sludge (Community "A")											
Influent	0	I,II, III	II,III	II	III	III	II,III	II,III	II,III	III	III
Effluent without Cl_2	0	III	0	0	0	0	0	0	0	0	0
Effluent with Cl_2	0	II	II	0	0	0	0	0	0	0	0
Trickling filter (Community "D")											
Influent	0*	I,II, III	I	I	I,III	I	I	I	I,III	I,II, III	I,III
Effluent	0*	I,II, III	ND	III	III	I,II, III	II,III	I,II, III	III	I,III	I,III
Receiving stream	ND	ND	ND	ND	ND	ND	ND	ND	ND	II	III

* Contains two nonpolio isolates.
ND Not done.
† Day of initial vaccination.
‡ Day of booster vaccination.

Poliovirus Type I, except during the initial sampling period, is absent from all subsequent influent samples taken from Community "A," whereas Type II, except for the initial sample and one instance during the booster sampling period, is absent from all other influent samples taken from Community "D." However, this virus is recovered from later samples of effluent and thus the discrepancy appears to be due to sampling variation. Poliovirus Type III persists in influent samples taken from both communities.

Of main interest is the disappearance of polioviruses from the effluent taken from the activated sludge treatment plant (Community "A") within six days following immunization, where no such effect could be noted following sewage treatment by trickling filtration (Community "D").

Chlorination of treated effluent of Community "A" did not appear to affect the recovery of poliovirus. It can be noted also that polioviruses Types II and III were recovered one-half mile downstream from the discharge of the trickling filter plant.

Table 3 compares the isolation of poliovirus types in H.Ep-2 and monkey kidney cell cultures. It can be observed that Types II and III were more often isolated in H.Ep-2 than in monkey kid-

Table 3—Comparison of isolation culture systems: sensitivity of rhesus monkey kidney (primary) and H.Ep-2 to the growth of poliovirus by type from sewage samples

Poliovirus type	RMK (only)	H.Ep-2 (only)	Both
I	8	3	6
II	1	10	7
III	5	11	12
Nonpolio*	2	0	0
Negative	6	3	21

* Not included in total.

ney cells, whereas the latter are more sensitive for Type I virus isolation.

Discussion

The effect of sewage treatment on virus survival has been the subject of several investigations.[4-6] Moreover, a study of poliovirus recovery after sewage treatment following mass oral poliovirus immunization was recently reported by Askew, et al.[11]

As confirmed in this study, all of these investigators found that the activated sludge process was superior to other conventional secondary sewage treatment methods in regard to removal of poliovirus. Our investigation was designed to provide a sewage sampling program whereby a simultaneous comparison of these two different treatment processes could be made soon after administration of live poliovirus vaccine. It was fortunate that just prior to the initial immunization no polioviruses were found in the effluent of either plant which might have obscured the fate of excreted vaccine virus.

It is readily observed that the activated sludge process prevents virus detection within three to six days after initial vaccine virus administration, although virus shedding was observed to continue for at least 56 days. These findings were confirmed after a second (booster) immunization was conducted.

The mechanism of virus removal has been attributed to either biological antagonism[5] or physical adsorption.[4] Although no definitive data is presented here, it would appear likely that the latter explanation has the greater merit, as indicated by the work of Roebeck, et al.[12]

One unusual aspect of this serial study is the virtual absence of Type I poliovirus from Community "A," (Table 2) three days after vaccine administration. Similarly a deficiency of recovery of Type II viruses was observed in Community "D." The data do not indicate sufficiently whether these discrepancies are the result of random sampling variation, prior immunological experience, or viral interference. The effect of chlorination on inactivation of poliovirus has been the subject of numerous investigations.[8] Although comparative observations were not available during our study (as chlorination was provided only in the activated sludge process plant) at the time of initial heavy viral input poliovirus was detected despite this procedure (Table 2).

The results indicate that it is important to consider the design of sewage treatment systems in terms of their ability to remove viruses, as well as for bacterial and other pollutional components, in order to reduce the dissemination of viral diseases. This is especially important when a new sewage treatment plant is contemplated or when an enlargement or addition is needed. In our study it was observed that the trickling filtration process was inferior to the activated sludge process in the removal of polioviruses. The trickling filtration plant released virus in sufficient quantity to be detected as far as one-half mile downstream from the point of discharge and on the basis of these findings would appear to offer inadequate public health

protection in regard to removal of viruses from sewage.

Summary and Conclusions

1. Activated sludge sewage treatment process was found to be far superior to trickling filtration for removal of trivalent oral poliovirus.
2. Excretion of such viruses was observed to continue for at least 56 days after vaccination.
3. Considerable variation in recovery of virus Types I and II was observed in the two different populations.
4. Chlorination did not eliminate virus recovery.

REFERENCES

1. Gelfand, H. M. The Occurrence in Nature of the Coxsackie and ECHO Viruses. Progr. Med. Virol 3: 193, 1961
2. Wiley, J., S., Chin, T. D.; Grovelle, C. R.; and Robinson, S. Enterovirus in Sewage During a Poliomyelitis Epidemic. J. Water Pollution Control Fed. 34:168, 1962
3. Kelly, S. M., Clark, M. E., and Coleman, M. B. Demonstration of Infectious Agents in Sewage. A.J.P.H. 45:1438, 1955
4. Clarke, N. A., Stevenson, R. F., Chang, S. L., and Kaller, P. W. Removal of Enteroviruses from Sewage by Activated Sludge Treatment. Ibid. 51: 1110, 1961.
5. Kelly, S., Sanderson, W. W., and Neidl, C. Removal of Enteroviruses from Sewage by Activated Sludge. J. Water Pollution Control Fed. 33:1056, 1961
6. Kelly, S., and Sanderson, W. W. The Effect of Sewage Treatment on Viruses. Sew. & Industr. Wastes 31,6 683, 1959
7. Gear, J. H. S. The Virus of Poliomyelitis: Its Distribution and Methods of Spread. J. Roy. San. Inst. 69,3 149, 1949
8. Mack, W. N., Mallman, W. L.; Bloom, H. H.; and Krueger, B. J. Isolation of Enteric Viruses. Sew. & Indust. Wastes 30,8 957, 1958
9. MacCallum, F. O., Cockburn, W. C., Smithard, F. H. R., and Wright, S. C. "The Use of Gauze Swabs for the Detection of Poliovirus in Sewers." In: Poliomyelitis, Papers and Discussions, Second International Polio Conference. Philadelphia, Pa., Lippincott, 1952, pp. 4184-4187.
10. Friedman, M., and Lieberman, M. Propagation of Virus in Tissue Culture Employing a Simple Maintenance Media Without Serum. Fed. Proc. Vol. 15: 587, 1956
11. Askew, J. B., Bott, R. F.; Leach, R. E.; and England, B. F. Microbiology of Reclaimed Water from Sewage for Recreational Use. A.J.P.H. 55,3: 453-462 (Mar.), 1965.
12. Roebeck, G. G.; Clarke, N. A.; and Dostal, K. A. Effectiveness of Water Treatment Processes in Virus Removal. J. Am. Water Works A. 54:1275, 1962.

Mr. Tucios is acting director, Division of Environmental Health, and Dr. Baker is director, Lake County Health Department (2307 Grand Avenue), Waukegan, Ill. Mr. Morris is superintendent, Lake County Public Works Department, Waukegan, Ill. Dr. Rosenbaum is chief, Virology Section, Naval Medical Research Unit No. 4, Great Lakes, Ill.

This paper was presented before a Joint Session of the National Association of Sanitarians, the Conference of State Sanitary Engineers, the Conference of Municipal Public Health Engineers, and the Engineering and Sanitation Section of the American Public Health Association at the Ninety Third Annual Meeting in Chicago, Ill., October 21, 1965.

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author) Naval Medical Research Unit No. 4 Great Lakes, Illinois	2a REPORT SECURITY CLASSIFICATION Unclassified 2b GROUP

3 REPORT TITLE

Effect of Sewage Treatment on Recovery of Poliovirus following Mass Oral Immunization

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final

5 AUTHOR(S) (Last name, first name, initial)

Theios, E.P., Morris, J.G., Rosenbaum, M.J., and Baker, A.G.

6 REPORT DATE	7a TOTAL NO. OF PAGES	7b NO. OF REFS
February 1967	6	12

8a CONTRACT OR GRANT NO	9a ORIGINATOR'S REPORT NUMBER(S)
b PROJECT NO MF 022.03.07-4016	RU 67.7
c	9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d	

10 AVAILABILITY/LIMITATION NOTICES

Unlimited distribution

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	Bureau of Medicine and Surgery Navy Department Washington, D.C.

13 ABSTRACT

Activated sludge sewage treatment process was found to be far superior to trickling filtration for removal of trivalent oral polioviruses.

Excretion of such viruses was observed to continue for at least 56 days after vaccination.

Considerable variation in recovery of virus Types I and II was observed in the two different populations.

Chlorination did not eliminate virus recovery.

DD FORM 1 JAN 64 1473

UNCLASSIFIED

Security Classification

UNCLASSIFIED

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Oral poliovirus immunization						
Poliovirus survival in sewage						

INSTRUCTIONS

1. **ORIGINATING ACTIVITY:** Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2a. **REPORT SECURITY CLASSIFICATION:** Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2b. **GROUP:** Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. **REPORT TITLE:** Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. **DESCRIPTIVE NOTES:** If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. **AUTHOR(S):** Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. **REPORT DATE:** Enter the date of the report as day, month, year; or month, year. If more than one date appears on the report, use date of publication.

7a. **TOTAL NUMBER OF PAGES:** The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b. **NUMBER OF REFERENCES:** Enter the total number of references cited in the report.

8a. **CONTRACT OR GRANT NUMBER:** If appropriate, enter the applicable number of the contract or grant under which the report was written.

8b, 8c, & 8d. **PROJECT NUMBER:** Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9a. **ORIGINATOR'S REPORT NUMBER(S):** Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9b. **OTHER REPORT NUMBER(S):** If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. **AVAILABILITY/LIMITATION NOTICES:** Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. **SUPPLEMENTARY NOTES:** Use for additional explanatory notes.

12. **SPONSORING MILITARY ACTIVITY:** Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13. **ABSTRACT:** Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS), (S), (C), or (U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. **KEY WORDS:** Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, roles, and weights is optional.

DD FORM 1 JAN 64 1473 (BACK)

UNCLASSIFIED