Serologic Survey of Rotavirus, Norwalk Agent and *Prototheca wickerhamii* in Wastewater Workers

C. SCOTT CLARK, PHD, CALVIN C. LINNEMANN, JR., MD, PETER S. GARTSIDE, PHD, JOHN P. PHAIR, MD, NEIL BLACKLOW, MD, AND CHESTER R. ZEISS, MD

Abstract: Analysis of paired sera from 48 wastewater workers and controls who reported gastrointestinal illness did not reveal any excess of seroconversions to Norwalk agent or to rotavirus. Inexperienced wastewater-exposed workers had higher levels of antibody to Norwalk agent than did experienced and control workers and those with high and medium aerosol exposure had higher titres than those in the low aerosol category. Analysis for *Prototheca* antibody titres was essentially negative. (*Am J Public Health* 1985; 75:83–85.)

Introduction

Workers engaged in sewer maintenance and wastewater treatment are exposed to a wide variety of routinely found disease-producing microorganisms. In spite of this exposure, literature searches have revealed little evidence of occupational health problems associated with wastewater pathogens.[1,2] In an earlier study of wastewater workers in Cincinnati, Chicago, and Memphis,[3] an increase in gastrointestinal illness was observed among inexperienced sewage-exposed workers when compared to experienced workers and controls; however, there was no consistent evidence of increased parasitic, bacterial, or viral infections as indicated by stool examinations, cultures, or antibody survey. The increase in illness occurred in the spring quarter and did not correspond to the enterovirus season.[3,4] Therefore the present study was planned to investigate any association between Norwalk agent or rotaviruses and the excess episodes of gastrointestinal illness previously detected among recently hired wastewater workers. In addition, sera were tested for an unusual algae, *Prototheca wickerhamii* which has been associated with wastewater environments.*

Methods

The previous study from which the present study derives was designed to detect differences in gastrointestinal infection rates and other indicators of health status between workers exposed to wastewater and control groups not so exposed. Total length of the study was 42 months.[3] Newly hired workers were recruited into the study when hired; experienced workers had a minimum of two years experience at the time of joining the study. The mean age of the workers was 38.4 years and 53 per cent were Caucasian. Control groups were highway maintenance workers in Cincinnati, waterworks employees in Chicago, and municipal gas and electric utility workers in Memphis. Additional demographic characteristics and other details of that study are described elsewhere.[3]

Work observation and air sampling were used to categorize aerosol exposure levels relative to other workers at the same facility. Relative wastewater exposure levels were determined by a combination of survey questionnaire and job observation.

Paired sera from a group of 48 workers (Table 1) with gastroenteritis were tested for antibody to rotavirus and to Norwalk agent.** Sera used were from routine quarterly collections before and after the reported illnesses.

Data relating to titre levels of antibody to rotavirus, worker group, season of year, age, race, type and degree of exposure, and job classification pertaining to the second sample tested from each worker were analyzed by analysis of covariance. Similar analyses were made for Norwalk agent antibody.

One hundred-eighty-eight serum specimens from 110 study participants were analyzed for IgG antibody to *Prototheca wickerhamii* algae.*** Single specimens were analyzed from experienced wastewater-exposed workers and paired specimens for inexperienced wastewater-exposed workers and controls. Selection of workers to be tested was based on availability of serum, race, and sex. Results were expressed as counts per minute and duplicate analyses were performed. Antibody response, age, race, sex, geographic location, exposure group, and economic status† were included in an analysis of covariance.

Results

Four-fold or greater titre level increases to rotavirus occurred in sera from two of 30 inexperienced wastewater workers and in two of the five experienced workers, indicating no significant differences among the worker groups. No differences were detected among the three work experience groups, or by race, wastewater exposure, aerosol exposure, job classification, or season in the analysis of covariance.

There was no significant difference in number of seroconversion to Norwalk agent among the three exposure groups. Four-fold or greater titre level increases to Norwalk

*Twenty-three cases of histologically-proven infection with Prototheca algae have been reported[5]; several were associated with wet environments, one of which was termed "sewage exposure." Prototheca algae have been found in waste stabilization ponds and in wastewater treatment plants.[6]

Address reprint requests to C. Scott Clark, PhD, Associate Professor, Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH 45267-0056. Dr. Linnemann is Professor of Internal Medicine, and Dr. Gartside is Associate Professor of Biostatistics and Environmental Health, both at the University of Cincinnati Medical Center. Dr. Phair is Director, Infectious Disease and Hypersensitivity Section, and Dr. Zeiss is Associate Professor of Medicine, both at Northwestern University Medical School, Chicago. Dr. Blackwell is Professor of Medicine, University of Massachusetts, Worcester. This paper, submitted to the Journal August 10, 1983, was revised and accepted for publication August 10, 1984.

**Antibodies to rotaviruses were detected by use of a micro enzyme-linked immunoassay (ELISA). An indirect sandwich technique was used with serologically related simian agent, SA-11 virus, as the antigen. This was grown in primary monkey kidney cells and extracted by treatment with flurocarbon. Antibody to Norwalk agent was determined by a radioimmunoassay.[7]

***IgG antibody to *Prototheca wickerhamii* algae was determined by a radioimmunoassay.[5,8]

†Economic status was defined as monthly household income ($1,000 units)/(# children + 1) with values <2 being assigned the "lower" category and values ≥2, "upper".

TABLE 1—Antibody Titres to Norwalk Agent in Single Serum Samples by Selected Variables

Variables†	Mean ± Standard Error*	N
All Workers*		
Location		
Cincinnati	3.07 ± 0.54	14
Chicago	2.89 ± 0.46	19
Memphis	1.67 ± 0.52	15
Experience (p = 0.03)		
Inexperienced	3.13 ± 0.38	30
Experienced	1.80 ± 0.88	5
Control	1.54 ± 0.54	13
Race (p = 0.06)		
White	2.22 ± 0.33	37
Black	3.73 ± 0.60	11
Sewage-Exposed Workers		
Wastewater Exposure		
Low	2.73 ± 0.60	15
Medium	3.25 ± 1.16	4
High	3.06 ± 0.58	16
Aerosol Exposure (p = 0.08)		
Low	1.63 ± 0.57	13
Medium	4.10 ± 0.65	10
High	3.42 ± 0.59	12
Race (p = 0.06)		
White	2.54 ± 0.43	26
Black	4.11 ± 0.73	9

*Values represent titre levels categorized as follows: <100 = 1, 100 = 2, 200 = 3, 400 = 4, 800 = 5, etc.

†Age, Job classification (laborer/other), and season not shown—Relationship not significant or important.

agent occurred in four of 30 inexperienced workers with gastroenteritis, in none of 13 controls, and in none of the five experienced workers.

Results of the analysis of the second sera for each worker (Table 1) indicated that the inexperienced workers had higher levels than the experienced and control workers (p = 0.03) and that those with high and medium aerosol exposure had higher titres than those in the low aerosol category (p = 0.08). Black workers had higher levels than White workers (p = 0.06).

Analysis of covariance for prototheca antibody for the three locations separately revealed that in Chicago experienced wastewater treatment workers and the waterworks employees had higher values than the inexperienced wastewater treatment plant workers (p = 0.003) but values for the control group were almost as high as those for the experienced wastewater worker, suggesting that wastewater exposure itself was not a cause of elevated titres. Race was also a significant co-variable in Chicago, with White workers having higher levels; economic status was a significant covariable only in Cincinnati with higher income level having higher average antibody levels. No differences were detected between the results of the analysis of the earlier sera tested and the later sera tested.

Discussion

Inexperienced wastewater-exposed workers had higher levels of antibody to Norwalk agent than did either the experienced or control groups after adjusting for differences in age and other co-variables. No evidence was found that associated rotavirus or *Prototheca wickerhamii* antibody levels with wastewater exposure.

An increase in symptoms of gastroenteritis among workers at six wastewater treatment plants in Sweden has been reported by Lundholm and Rylander.[9] Our later analyses of viral serosurvey data according to exposure level[10] have also suggested that the subgroups of inexperienced wastewater workers with higher exposure to wastewater and bacteria aerosols had higher antibody levels to a group of enteroviruses.†† However, the fact that antibody level was not related to length of wastewater exposure in the later analyses adds support to the conclusions reached in the previous study[3] that the usual occupational conditions at modern wastewater treatment plants do not pose a major risk of virus infection. A similar conclusion was reached for the risk of viral infection among workers at a wastewater spray irrigation site.[11] A comprehensive retrospective study of Berlin sewer workers by Anders[12] did not detect any health problems associated with sewage pathogens. The few situations where adverse health effects have been detected were usually associated with sewage farming with untreated wastes or with other contact with essentially raw sewage.[13–15]

Although most studies of routine exposures at wastewater facilities reveal little if any risk of disease associated with wastewater exposure, a number of studies continue to appear showing some evidence of increased risk of infections with hepatitis A,[16–18] Leptospirosis,[19–21] and parasites.[23,24] These reports usually stem from investigations prompted by apparent outbreaks of disease among wastewater workers and demonstrate that under certain conditions infections appear to be associated with wastewater employment. These reports and our findings with inexperienced workers point up the importance of efforts to minimize contact with wastewater and to continue to promote basic hygienic principles.

††Further data available on request to authors.

REFERENCES

1. Hickey JLS, Reist PC: Health significance of air borne microorganisms from wastewater treatment process: II. health significance and alternatives for action. J Water Poll Cont Fed 1975; 47:2758–2773.
2. Clark CS, Cleary EJ, Schiff GM, Linnemann CC Jr, Phair JP, Briggs TM: Disease risks of occupational exposure to sewage. J Environ Engineer Div Am Soc Civil Engineers 1976; 102:375–388.
3. Clark CS, Van Meer GL, Linnemann CC Jr, Bjornson AB, Gartside PS, Schiff GM, Trimble SE, Alexander D, Cleary EJ, Phair JP: Health Effects of Occupational Exposure to Wastewater. *In:* Pahren H, Jakubowski W (eds): Wastewater Aerosols and Disease. Proceedings of a symposium, US Environmental Protection Agency, Cincinnati, Ohio, December 1980, EPA-600/9-80-02-8.
4. Clark CS, Linnemann CC Jr, Van Meer GL, Schiff GM, Gartside PS, Bjornson AB, Cleary EJ, Phair JP, Buncher CR, Alexander DL, Trimble SE, Barnett BC: Health Risks of Human Exposure to Wastewater. U.S. Environmental Protection Agency, Health Effects Research Laboratory, PB 81-143406, March 1981.
5. Venezio FR, Lavoo E, Williams JE, Zeiss CR, Caro WA, Mangkornkanok-Mark M, Phair JP: Progressive cutaneous protothecosis. Am J Clin Pathol 1982; 77:485–493.
6. Cooke WB: Studies in the genus prototheca: I. toxonomy. J Elisha Mitch Sci Soc 1968; 84:217–220.
7. Blacklow NR, Cukor G: Viral gastroenteritis agents. *In:* Lennette E, Balows A, Hausler WJ, Truant JP (eds): Manual of Clinical Microbiology, 3rd Ed. Washington, DC: American Society for Microbiology, 1980.
8. Phair JP, Williams JE, Bassaris HP, Zeiss CR, Morlock BA: Phagocytosis and algicidal activity of human polymorphonuclear neutrophilis against *Prototheca wickerhamii*. J Infect Dis 1981; 144:72–76.
9. Lundholm M, Rylander R: Work-related symptoms among sewage workers. Br J Ind Med 1983; 40:325–329.
10. Clark CS, Bjornson HS, Linnemann CC Jr, Gartside PS: Evaluation of Health Risks Associated with Wastewater Treatment and Sludge. Cincin-

nati: US Environmental Protection Agency, Health Effects Research Laboratory, Final Report, Cooperative agreement CR 807357, July 1984.
11. Linnemann CC Jr, Jaffa R, Gartside PS, Scarpino PV, Clark CS: Risk of infection associated with a wastewater spray irrigation system used for farming. JOM 1984; 26:41–44.
12. Anders W: The Berlin sewer workers. Zeitschrift for Hygiene 1960; 1:341–371. (English translation by Ralph E. Oesper, University of Cincinnati.)
13. Central Public Health Engineering Research Institute (CPHERI): Health status of sewage farm workers. Technical Digest, No. 12, Nagpur, India, May 1971.
14. Nityanada K: Leptospirosis-Serological Survey of Occupational Groups in Ceylon. J Trop Med Hyg 1967; 70:250–254.
15. Fuchs GHP: Problems of occupational leptospirosis in so-called dirty occupations. Zbl Bakt I Abt Orig 1960; 180:549–561. (Excerpta Medica 1961, 1971).
16. Skinhoj PF, Hollinger FB, Hovind-Hougen K, Lous P: Infectious liver diseases in three groups of Copenhagen workers: correlation of hepatitis A infection to sewage exposure. Arch Environ Health 1980; 36:139–143.
17. Dixon FR, McCabe LJ: Health aspects of wastewater treatment. J Water Poll Cont Fed 1964;36:984–989.
18. Kosatsky TB: Hepatitis A among Sewage-treatment Workers, Anchorage, Alaska. Epidemic Intelligence Service, 32rd Annual Conference, April 18–22, 1983, Centers for Disease Control, Atlanta, Georgia (Abstract).
19. Muraschi TF, Harris AH: Leptospirosis, past and present in New York state. NY State J Med 1961; 61:2750–2756.
20. Heath CW Jr, Alexander AD, Galton MM: Leptospirosis in the United States. N Engl J Med 1965; 273:857–864.
21. Broom JC: Leptospirosis in England and Wales. Br Med J 1951; 689–697.
22. Doby JM, Duval JM, Beaucournu JS: Amoebiasis, an occupational disease of sewer workers? La Nouvelle Presse Medicale 1980; 9:532–533. (English translation by Kettering Library, University of Cincinnati.)
23. Knobloch J, Bialek R, Hasemann J: Intestinal protozoal infestation in persons with occupational sewage contact. Dtsch Med Wochenschr 1983; 108:57–60. (English translation by Kettering Library, University of Cincinnati.)

ACKNOWLEDGMENTS

We wish to acknowledge support of the many workers who participated in this study and the organizations for whom they worked. In addition, we acknowledge the capable efforts of Darryl Alexander, Sally Trimble, and Gretchen Van Meer, PhD, in the earlier study which produced the serum bank used in this study and to Jane Onslow for her efforts during both studies. The encouragement and advice of the US Environmental Protection Agency project officers, Walter Jakubowski and Herbert Pahren, have also been appreciated.

This work was supported by US Environmental Protection Agency Grant No. R 803643 and US Environmental Protection Agency Cooperative Agreement No. CR 807357-01, both to the University of Cincinnati, and by US Environmental Protection Agency Cooperative Agreement No. CR 810803010 to the University of Massachusetts.

Vehicular Carbon Monoxide Screening: Identification in a Cross-Cultural Setting of a Substantial Public Health Risk Factor

ROBERT L. WILLIAMS, MD

Abstract: A community program of screening and education for prevention of vehicular carbon monoxide (CO) poisoning among a high-risk population in a cross-cultural setting is presented. The program was developed after two infant deaths in separate incidents of vehicular CO poisoning. The results of the screening show 18.6 per cent of vehicles exceeding the Environmental Protection Agency eight-hour standard for CO exposure, and 2.6 per cent exceeding the one-hour standard. Extension of such programs to other high-risk populations is recommended. (*Am J Public Health* 1985; 75:85–86.)

Introduction

The medical literature abounds with descriptions of carbon monoxide poisoning and its management.[1–8] The present study describes a program of primary prevention in high risk situations of vehicular exposure in a cross-cultural setting.

Description of Setting

The Crownpoint Service Unit of the Indian Health Service provides health care through its hospital, clinics, and public health activities to 12,000 Navajo Indians located in a 4,500 square mile area of northwest New Mexico. The beneficiary population's unemployment rate approximated 65–70 per cent in 1982 and per capita income in 1983 was $2,250*. Many of the residents follow a predominantly traditional lifestyle and culture, with a large percentage speaking little or no English. Current scientific concepts, including those related to illness causation, are incompletely understood or accepted by the traditional culture.

In March 1982, two Navajo infants suffered sudden death in separate episodes while riding in vehicles, and were brought to the emergency room at the Crownpoint Hospital. Carbon monoxide (CO) poisoning was identified as the cause in both cases, and the interiors of both vehicles were shown to have toxic ambient levels of carbon monoxide. In response to this, a community carbon monoxide screening and education program was developed.

Methods

Following a series of educational presentations to health care providers, the community education program was begun. This consisted of three aspects: 1) an ongoing presentation by the health educator in the clinic waiting room; 2) presentations given at community meetings, individual homes, and field clinics by community nurses and community health representatives; and 3) radio and newspaper discussions and announcements. A device for measuring ambient CO levels** was then taken to 14 prominent locations throughout the area, and made available for free testing of vehicular interiors. The testing was performed in closed

Address reprint requests to Robert L. Williams, MD, Navajo Area, Indian Health Service, US Public Health Service, PHS Indian Hospital, Crownpoint, NM 87313. This paper, submitted to the Journal May 21, 1984, was revised and accepted for publication August 27, 1984.

*Navajo Tribe, Department of Economic Planning.

**Energetics science, Ecolyzer (R), CO analyzer, series 2000, model A1514.