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EPA

Waterborne Disease Outbreaks

Selected Reprints of Articles on Epidemiology, Surveillance, Investigation, and Laboratory Analysis

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Edited by
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Notice

The opinions presented in this document are those of the authors and do not necessarily reflect the views of the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Preface

This volume is dedicated to the engineers, sanitarians, microbiologists, epidemiologists, and other health officials who have labored throughout this century to improve the quality of our drinking water. Because of their endeavors, we in the United States and most developed countries now enjoy a quality of life in which waterborne disease outbreaks are no longer a constant threat or danger. This status has been achieved by the tireless efforts of many and often with little public recognition.

Although the incidence of waterborne disease has been drastically reduced in the United States, we must not forget that infectious diseases still remain a threat for billions of people in the developing world where water supplies are not as well protected from sewage and may be inadequately treated. The lessons we have learned are important for reducing the worldwide incidence of waterborne diseases. We must also continue our vigilance because a residual number of outbreaks still occur in the United States, and this indicates the vulnerability of our water supplies.

We have learned that many of our water sources are inadequately protected and treated to prevent the transmission of giardiasis. The waterborne transmission of this disease was first reported in the United States in 1965 and has increasingly been reported since 1971. *Giardia* is now the most commonly identified pathogen in waterborne outbreaks. Contaminated surface water is a significant source of infection for giardiasis, and almost half of the outbreaks of waterborne giardiasis have occurred in surface water systems where the only treatment was disinfection. In addition to *Giardia*, a number of new etiologic agents have been recognized in recent years and as laboratory analysis and investigation procedures improve, more agents are likely to be identified. Some of the newly recognized waterborne pathogens include *Cryptosporidium*, *Campylobacter*, *Yersinia*, hemorrhagic *E. coli* 0157:H7, rotavirus, and Norwalk agent. This volume also describes a chronic diarrhea for which an agent has yet to be identified despite extensive laboratory analyses.

Only a fraction of the waterborne outbreaks that occur in the United States are recognized, investigated, and reported, and in only half of the reported waterborne outbreaks was an etiologic agent identified. We must improve the investigation and surveillance of waterborne disease to identify the causes of these outbreaks and the etiologic agents. The identification of *Giardia* as an important waterborne pathogen stimulated the research which has identified the types of filtration, operating parameters, and disinfection levels necessary for removal and inactivation of this pathogen. Water treatment research is now being conducted to obtain similar information for *Cryptosporidium*.

The emphasis of this volume is on drinking water systems and the prevention of infectious waterborne diseases. We must improve surveillance, reporting, epidemiologic investigations, and laboratory analyses, and we cannot rest on past accomplishments. Continuing microbiological challenges are a reminder that our task to eliminate waterborne illness is yet unfinished.

G.F.C.

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Acknowledgments

A number of previously published articles have been reprinted in this volume to supplement information contained in a separate publication, *Methods for the Investigation and Prevention of Waterborne Disease Outbreaks.* These articles provide examples of outbreak investigations and surveillance activities. They have been reprinted to illustrate various aspects of epidemiologic methods and engineering principles and compiled as a convenience to those investigators with limited access to library facilities. Articles have also been included on new clinical procedures for viral and protozoan pathogens as these are discussed only briefly in the methods volume. The contributions of the authors of the journal articles are gratefully acknowledged.

Permission to reprint these articles has been provided by: Journal of Epidemiology and Community Health, American Journal of Public Health, Journal of Environmental Health, American Journal of Epidemiology, Annuals of Internal Medicine, The New England Journal of Medicine, Lancet, Journal American Water Works Association, and Journal of Clinical Microbiology.

This report has been reviewed by the U.S. Environmental Protection Agency and approved for publication.

I. Introduction

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Review article: Research methods in epidemiology, III

Epidemiology in search of infectious diseases: methods in outbreak investigation

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The value of epidemiology in the investigation of incidents of infectious disease in the UK does not seem to be always readily appreciated within microbiology[1 2] or environmental health.[3] Yet because microbial disease is a result of the interaction of the agent, the host and environmental factors[4] neither laboratory diagnosis nor environmental inspection are of themselves sufficient for successful investigation and control. Simply isolating microbes from the environment, even if appropriate samples are available, cannot distinguish between a reservoir of an agent and the outbreak source or cross contamination from the reservoir or source. Microbiologically plausible explanations of outbreaks may be wrong, but it is often difficult to persuade investigators of the need to test such hypotheses.

On the other hand, whilst epidemiology may have evolved from the study of infectious disease epidemics, academic epidemiology seems in the main to have passed infectious diseases by in the UK. This is regrettable as there is still the need to consolidate the foundations of a section of public health medicine which has refused to disappear.[5] This review, in following the steps taken in an outbreak investigation,[6] draws attention to both the usefulness of epidemiology and to regularly recurring methodological problems which may be encountered.

Describing the outbreak

The classical "shoe leather" approach to infectious disease incidents is the detailed investigation of individual cases in the light of the well established microbiological features of the particular infection and a confidence in the possible routes of transmission.[7] The background incidence of infectious diseases in the UK is usually well known and is sufficiently low not to require sophisticated statistical methods of cluster analysis[8] to recognise a local outbreak. However, with newly recognised infections or diseases presumed to be caused by an unknown infectious agent, surveys of disease incidence or laboratory isolation rates may be necessary to confirm that an outbreak has occurred. The methodological problems encountered are similar to those found in investigating clusters of cancers.[8]

The cases first reported in an outbreak are usually only a small and sometimes biased sample which may mislead, rendering control measures ineffective. Identification and definition of the exposed population is necessary to enable thorough case finding to be carried out. Paradoxically, however, a single case which does not fit the usual characteristics of time, place and person may provide the most valuable evidence of the source of infection.[7]

Data from cases are characterised by time, place and person.[6] The epidemic curve, a plot of the number of cases over time of onset of illness, is the most useful and immediate means of assessing the type of outbreak. In point source outbreaks in which all cases are exposed at a given time, onset of symptoms of all primary cases will cluster within the range of the incubation period. For each organism there is a characteristic range within which infecting dose[9] and portal of entry, as well as host factors[10] (eg, age, immunosuppression) give rise to individual variability. In outbreaks propagated from person to person the occurrence of cases will be spread over several incubation periods with peaks at intervals of the incubation period. In larger community outbreaks of diseases spread by person to person the epidemic curve is usually smoother and the peaks at the generation time of the new cases less obvious. Despite their almost universal use in outbreak investigations, there has been little formal evaluation of their use in practice. Sartwell in 1950 established that the frequency distribution of incubation periods of infectious diseases of both long and short incubation

period followed a log normal curve.[11] He suggested the use of the dispersion factor, the antilogarithm of the logarithmic standard deviation, to measure variation in incubation periods. Such analyses may be used to distinguish between point source and continuing source outbreaks, estimate the data of a common exposure, determine time of maximum infectiousness, help explain pathogenic mechanisms,[12] identify the occurrence of secondary spread, and evaluate control measures. However, Armenian and Lilienfeld have claimed the initial efforts of Sartwell "have not been followed by any systematic review of the data on incubation periods".[13]

Developing a hypothesis

The traditional distinction between descriptive (hypothesis generating) and analytical (hypothesis testing) studies has been criticised by Rothman,[14] a criticism which holds true in outbreak investigation. At the simplest level epidemiological investigation may be described in two stages: firstly, generating a hypothesis of causation from descriptive data analysed by time, place and person, and secondly, testing that hypothesis by a case-control or cohort study. But in almost all outbreaks competing a priori hypotheses are voiced at the outset by public health investigators, patients, employers, food producers, etc. Some of these hypotheses may be excluded by the "descriptive" data. These descriptive data in turn may stimulate alternative hypotheses. Indeed, as in clinical investigation of an individual patient, this process of hypothesis generation and refutation goes on continuously as new cases are being interviewed, and challenges the rigid dichotomy of descriptive versus analytic. For example, in an outbreak of *Salmonella ealing*, cases were mainly infants who were discovered to have been fed on a single brand of milk formula.[15] Comparison with a group of well infants showed a significant difference in use of this brand. Was this comparison testing the hypothesis, or could such a design only firm up the hypothesis—the hypothesis seemingly was generated on the same set of cases as it was "tested"? In such outbreaks a priori hypotheses are formulated on the prior knowledge of the epidemiology of salmonellas and, in this case, on the observation of the age distribution of cases. The search for a common milk formula fed to the infants followed a prediction based on the deduction that the most likely vehicle of infection in infants was formula feed.

In some outbreaks the descriptive epidemiology suggests hypotheses for which there are no biological explanations[16 17] (eg, a cluster of three cases of aplastic anaemia in children[18]) or which go against the accepted microbiological theory (eg, an outbreak of shingles[19 21]). In the practice of infectious disease control, the framework of proven microbiological causes and modes of transmission enables statistical data to be interpreted confidently. Once outside known causal pathways statistical associations are difficult to interpret and public health action, like the clinical diagnosis of an individual patient, usually is taken on the basis of clinical experience and instinct.

Hypothesis testing

When hypotheses are formally tested the epidemiological techniques usually used are retrospective cohort and case-control studies. In certain outbreaks, eg, food poisoning outbreaks in institutions or following receptions, it is usually possible to identify retrospectively and follow up the whole population to identify simultaneously exposures and disease status. (This itself may lead to biases which are described below.) Comparison of attack rates in exposed and unexposed (eg, food specific attack rates) or exposures in cases and controls will often implicate the source. Paradoxically, when a vehicle is universal, eg, tap water, it may be impossible to demonstrate an association with illness unless there is a dose response (which is less likely if the vehicle of infection is heavily contaminated). In addition to universal problems of matching, confounding and appropriate analysis there are regularly recurring problems of design and implementation in outbreak investigations.

SAMPLING BIAS

Sampling may not be required at all as the size of the population at risk or the number of cases is usually small. However the corollary of this is that the power of possible studies in outbreak investigations cannot usually be controlled by the investigators. Nevertheless, the speed at which such studies can be done by experienced investigators, and the relatively low cost of the studies, as well as the potential benefits of demonstrating an association between infection and a possible source, mean that studies of low power will usually still be worth carrying out.

Diagnostic suspicion bias[22] in case selection is always a problem when a possible vehicle of infection is widely publicised. For example, people who have eaten a food believed to be the source of an outbreak of botulism may present with neurological symptoms unrelated to botulism. When laboratory confirmation of diagnosis is not possible the only safeguard is to choose cases which meet the strictest clinical case definition (and therefore who are at the most severe end of the disease spectrum).

In case control studies the selection of controls always poses a problem of practicality versus validity. Sampling frames commonly used to select controls

include general practitioner age/sex registers, hotel and reception guest lists, family members of cases, neighbours of cases, and persons investigated by the laboratory but who were negative for the disease in question. When diagnostic access bias[22] is considered potentially important (eg, cases of enteric infections are only recognised in the main if they have general practitioners who regularly recommend faecal sampling), it is important to match for neighbourhood of residence, or use patients investigated by laboratories but found to be negative, since failure to do so may lead to a spurious association with, say, a restaurant in the neighbourhood of the "keen" general practitioner. However, using general practitioner lists may result in overmatching. Obtaining controls from general practitioner registers is laborious and slow, and in our experience about 5–10% of general practitioners are reluctant to cooperate.

Increasingly, acquaintances nominated by cases are used as controls[15] although the representativeness of such groups and possible biases they may introduce have not been evaluated systematically. It is possible, for example, that if cases know that a particular exposure is the source of the outbreak their nomination of controls will be influenced. Additionally, the case may know the exposure history of several acquaintances and reveal this with their names to the interviewer, so possibly influencing the interviewer's choice of controls. A protocol to avoid this may be derived, although in practice there is the danger of a conflict of priorities when local investigators, eg, environmental health officers with their own local responsibilities for outbreak investigation, are called upon to carry out the national protocol.

Misclassification of controls can be avoided by asking all the individuals in the cohort or control group about symptoms over the appropriate time period so that those who may be unrecognised cases can be excluded or reclassified. The feasibility of serotesting, swabbing or otherwise testing controls to exclude or reclassify asymptomatic infected persons should always be considered. However, few well people are willing to give a sample of faeces, and obtaining blood samples from well children is difficult and may be considered by some clinicians to be unethical.

MEASUREMENT BIASES

In all but the smallest outbreaks data are collected by questionnaire, but often practicalities require groups to be interviewed by a combination of telephone, mail, face to face and proxy interviews. The sensitivity and specificity of questionnaires is seldom considered. In one study food consumption recalled by people 2–3 days after luncheon was compared with that observed at the time and recorded on video tape.[23] Only four of 32 attendees made no errors in reporting. The sensitivity of the food consumption histories questionnaire was 87.6% and the specificity 96.1%. Thirteen percent of the respondents reported eating "dummy" food items listed on the questionnaire but not served at the luncheon. Such errors in recall can be reduced by providing background details of events, good questionnaire design, and making use of other sources to check data such as diaries, menus, discussion with relatives, etc. Validation of patient histories is often not possible in practice due to the nature of the exposures, the lag between exposure and investigation and the urgency of investigation. However, random errors in recall are unlikely to give rise to false associations between illness and, for example, a food item. Rather they will reduce the power of a study to identify the true vehicle of infection. For some types of data, such as history of immunization, patient or parent recall are particularly inaccurate and validation of vaccine history from medical records is required.[24]

Loss of cases or controls from the study because of refusal to be interviewed or failure to trace patients is much less a problem in outbreak investigation since the affected population is usually eager to know the cause, and response rates should approach 100%. Throughout the course of their illness cases will have gone over in their minds possible exposures and their recall may be biased by their own preconceptions or by press and media speculations.

Cases will often have been interviewed on many occasions (by general practitioners, environmental health officers, community physicians) before an analytical study is carried out, and this may have introduced bias from suggestions made by interviewers, as well as prompting a more detailed recall. Family members may have suggested likely sources of infection to the cases. Patients may have anticipated the view of the interviewer if the suspected cause of the outbreak has been learned from local media reports and they may alter their answers accordingly. These biases can often be anticipated and can sometimes be assessed in analysis. For example, in a suspected waterborne outbreak of giardiasis in Reno, Nevada, considerable local publicity occurred before the case-control study was conducted.[25] However, because of the long incubation period of giardia many new cases became ill several days after the water supply was treated and so media attention persisted and water was still considered suspect. However, increased water consumption was only associated with cases exposed before the water supply was treated. Bias from media reports was likely to have affected later cases also but no association was found.

A major methodological problem is the bias which may result from the interviewer knowing the disease status of the person and having his own suspicion or prejudice as to the source. Selective histories may be taken in which the patient with salmonella is only asked in detail about a particular food. Blinded interviewing is rarely possible since resources are limited (most outbreaks are investigated by only one or two people), and indeed exposure history is usually obtained at the same time as disease history. Professionalism in the technique of interviewing, and use of a structured questionnaire are the only safeguards. Controls, who anyway have no special reason to recall certain exposures, are usually interviewed several days after cases, which adds to problems of accuracy and detail of recall. To help overcome this problem, questionnaires may include memory jogging points related to notable events, etc. It is important that the controls are interviewed as carefully and thoroughly as the cases were.

Conclusion

Do epidemiological studies in outbreak investigations have to be "quick and dirty" or can they be "quick and clean"? They must be quick. There is a need to develop and evaluate epidemiological methods in outbreak investigation so that they are both practical and sound. Epidemiological standards for the practice of the newly re-established discipline in public health medicine – communicable disease control – must be developed so that attributed causes of outbreaks are not left to be a "matter of opinion".[26]

References

[1] Anonymous. Stanley Royd: the epidemiological lesson. *Br Med J*, 1986; **292:** 644 5.

[2] Powell DEB. Salmonella enteritidis. *Lancet* 1989; i: 99.

[3] North R. *A failure of government. A report on the risk of food poisoning from eggs and egg products.* Bradford: Foodguard Publications, 1989.

[4] Brachman PS. The control of infectious diseases. In: Holland WW, Detels R, Knox G, eds. *Oxford textbook of public health,* Vol 2. Oxford: Oxford University Press, 1985: 8 18.

[5] Evans AS. The eradication of communicable diseases: myth or reality? *Am J Epidemiol* 1985; **122:** 199 207.

[6] Gregg MB. The principles of an epidemic field investigation. In: Holland WW, Detels R, Knox G, eds. *Oxford textbook of public health,* Vol 2. Oxford: Oxford University Press, 1985: 284 99.

[7] Galbraith NS. The application of epidemiological methods in the investigation and control of an acute episode of infection. In: Holland WW, Detels R, Knox G, eds. *Oxford textbook of public health,* Vol 4. Oxford: Oxford University Press, 1985: 3 21.

[8] Openshaw S, Craft AW, Charlton M, Birch JM. Investigation of leukaemia clusters by use of a geographical analysis machine. *Lancet* 1988; i: 272 3.

[9] Blaser MJ, Newman LS. A review of human salmonellosis I. Infective dose. *Rev Infect Dis* 1982; **4:** 1096 106.

[10] Khoury MJ, Flanders WD, Greenland S, Adams MJ On the measurement of susceptibility in epidemiologic studies. *Am J Epidemiol* 1989; **129:** 183 90.

[11] Sartwell PE. The distribution of incubation periods of infectious disease. *Am J Hyg* 1950; **51:** 310 18.

[12] Sartwell PE. The incubation period and the dynamics of infectious disease. *Am J Epidemiol* 1966; **83:** 204 16.

[13] Armenian HK, Lilienfeld AM. Incubation period of disease. *Epidemiol Rev* 1983; **5:** 1 15.

[14] Rothman KJ. *Modern epidemiology.* Boston, Toronto: Little Brown, 1986.

[15] Rowe B, Begg NT, Hutchinson DN, *et al.* Salmonella ealing infections associated with consumption of infant dried milk. *Lancet* 1987; ii: 900 3.

[16] Schwartz RS, Callen JP, Silva J. A cluster of Hodgkin's disease in a small community. *Am J Epidemiol* 1978; **108:** 19 26.

[17] Gordis L. Challenges to epidemiology in the next decade. *Am J Epidemiol* 1988; **128:** 1 9.

[18] Morgan GJ, Palmer SR, Onions D, Anderson M, Cartwright RA, Bentley DP. A cluster of three cases of aplastic anaemia in children. *Clin Lab Haematol* 1988; **10:** 29 32.

[19] Palmer SR, Caul EO, Donald DE, Kwantes W, Tillett H. An outbreak of shingles? *Lancet* 1985; ii: 1108 11.

[20] Peto TEA, Gilks CF, Juel-Jensen BE. Clusters of shingles. *Lancet* 1985; ii: 1433.

[21] Palmer SR, Tillett H. Shingles clusters. *Lancet* 1986, i: 273 4.

[22] Sackett DL. Bias in analytic research. *J Chronic Dis* 1979; **32:** 51 63.

[23] Decker MD, Booth AL, Dewey MJ, Fricker RS, Hutcheson RH, Schaffner W. Validity of food consumption histories in a foodborne outbreak investigation. *Am J Epidemiol* 1986, **124,** 859 63.

[24] Orenstein WA, Bernier RH, Dondero TJ, *et al.* Field evaluation of vaccine efficacy. *Bull WHO* 1985; **63:** 1055 68.

[25] Navin TR, Juranek DD, Ford M, Minedew DJ, Lippy EC, Pollard RA. Case control study of waterborne giardiasis in Reno, Nevada. *Am J Epidemiol* 1985; **122:** 269 75.

[26] House of Commons Agriculture Committee. *Salmonella in eggs. First Report,* Vol 2 (Minutes of evidence and Appendices). London: HMSO, 1989.

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II. Disease Surveillance

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Waterborne Disease in Colorado: Three Years' Surveillance and 18 Outbreaks

RICHARD S. HOPKINS, MD, PAMELA SHILLAM, BS, BARRY GASPARD, BS, LINDA EISNACH, MPA, AND RICHARD J. KARLIN, PE

Abstract: The Colorado Department of Health conducted intensive surveillance for waterborne diseases during the three-year period July 1, 1980–June 30, 1983. Eighteen outbreaks of waterborne illness were investigated. Outbreaks involved from 15 to 1,500 ill persons. *Giardia lamblia* was confirmed or suspected as the agent in nine outbreaks, rotavirus in one, and no agent could be identified in eight. Seventeen outbreaks occurred on surface-water systems; none of these had adequate chemical pretreatment and filtration. Investigation of water systems exhibiting positive coliform results during the first year detected no outbreaks. Activities important to effective surveillance included educational outreach programs to local health agencies, physicians and the public, and the designation of one individual to whom all water-related public complaints and health department inquiries were directed. (*Am J Public Health* 1985; 75:254–257.)

Introduction

There has been a steady increase in the number of reported waterborne disease outbreaks occurring in the United States since the early 1960s. The average annual number reported for 1971–72 was 32; from 1980–82 the average was 41.1.[1] The actual number of outbreaks that occur is certainly greater than this.

Over a three-year period prior to June 1980, six waterborne outbreaks were reported in Colorado but about 20 additional clusters of gastrointestinal illness was suspected to be waterborne. Energy development, tourism, and population growth combine to place stress on water systems that typically have relied on protected or remote surface sources of supply with marginal treatment.

Prior to June 1980, the Colorado Department of Health had a passive waterborne outbreak surveillance system, and responsibility for follow-up of water-related complaints was divided among two Sections of the Water Quality Control Division and the Communicable Disease Control Section.

From June 1980 through June 1983, a field epidemiologist was made responsible for improving the detection of waterborne illness.* The project was housed in the Communicable Disease Control Section, Division of Disease Control and Epidemiology, which receives reports of communicable disease cases from the state's 63 counties.

Methods

In the first year, surveillance was expanded to include a daily review of coliform results from the required testing of community and non-community water systems. When positive samples were received, physicians, pharmacists, public health nurses, and schools in areas served by the affected water systems were promptly surveyed by telephone for evidence of an outbreak of gastrointestinal disease, without waiting for check samples.

Throughout the project, engineers in the Water Quality Control Division supplied information from their inspections of water systems that indicated a potential hazard, and investigated water systems when a waterborne outbreak was suspected. In the second and third years of the project, questionnaires were mailed to all persons with laboratory-confirmed giardiasis reported to state or local health departments. Two or more laboratory-confirmed giardiasis case reports linked by time and place or two or more independent citizen reports from the same area of excessive illness suspected of being waterborne prompted an active surveillance effort as described above.

In May 1981, an education program directed toward county health officials was instituted; this included formal presentations to 10 of the 13 largest county health departments. Public health nurses and sanitarians were encouraged to report to one another and to the project office unusual occurrences of disease and problems discovered in local water systems.

Public awareness about waterborne diseases was heightened through media coverage of outbreak investigations. Complaints from the public about illness or poor quality drinking water were directed from the engineering sections to the waterborne disease project.

The state health department's bi-weekly Colorado Disease Bulletin (circulation 4000) was used to inform private physicians and local health agencies of the project and to solicit reports of potential waterborne disease problems. Outbreak investigations were described in the Bulletin to maintain interest in the project.

Community surveys conducted in the investigation of all potential waterborne outbreaks sought information about various symptoms, onset dates, duration, amount of water consumption prior to illness, and water supply source. The standard water question was: "How many 8-ounce glasses of unheated tap water, or drinks made up with unheated tap water, did you drink on an average day before (*date of outbreak*)?" These surveys were usually conducted via telephone, using modified random digit dialing techniques when a water system's distribution area approximated an area served by all or part of one telephone exchange. Samples were randomized and consisted of at least 40 households. In several investigations dealing with persons who had dispersed from a common site, mailed questionnaires were used.

*US Environmental Protection Agency contract #68-03-2927, June 1980–June 1982.

Address reprint requests to Dr. Richard S. Hopkins, Colorado Department of Health, Communicable Disease Control Section, Division of Disease Control and Epidemiology, 4210 E. 11th Avenue, Denver, CO 80220. Authors Shillam, Gaspard, and Eisnach are also affiliated with that Division; author Karlin is with the Drinking Water Section, Water Quality Control Division, CDH. This paper, submitted to the Journal May 7, 1984, was revised and accepted for publication October 15, 1984.

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An outbreak was considered to be waterborne when a positive dose-response relationship between amount of water consumed and attack rate could be demonstrated and when there was a significant difference in attack rate between persons drinking from the implicated supply and those drinking no water from the implicated supply. The project protocol required a minimum of six stools from ill persons for examination and identification of pathogenic agents including bacteria (*Salmonella, Shigella, Campylobacter* and *Yersinia*), intestinal parasites, and viruses and the same number from well controls. When a viral outbreak was indicated by symptom profile, the protocol also required collection of paired sera from a minimum of six ill and six well individuals. Viral specimens were frozen and sent to the Health Effects Research Laboratory, US Environmental Protection Agency (EPA), Cincinnati, Ohio. Bacteriological and parasitological laboratory analyses were conducted at the Colorado State Laboratory by standard methods. Because of late notification of outbreaks, not all indicated specimens could be collected in all outbreaks.

In suspected waterborne outbreaks, water samples were collected and examined for total coliforms and fecal coliforms. In outbreaks of suspected giardiasis, 1,000 gallons each of treated and untreated water were filtered through a one micron filter and the filter examined for the presence of *Giardia* cysts by Dr. Charles Hibler of Colorado State University.

Results

Eighteen outbreaks of gastrointestinal disease were shown to be waterborne. An additional 10 outbreaks were strongly suspected of being waterborne. Twelve reports of outbreaks were sufficiently suspicious to warrant significant further investigation, but most were shown to be due to person-to-person spread, not waterborne.

Etiology of Outbreaks

Nine of the 18 waterborne outbreaks are considered to have been caused by *Giardia lamblia*, although confirmation of the responsible agent is not equally good in all nine (Table 1). One was associated with rotavirus.[2] No agent was identified for the remaining eight outbreaks. In four of these outbreaks (#1, 2, 3, 6) which resembled Norwalk virus gastroenteritis clinically, epidemiologic investigation began at a time when no new cases were occurring and a decision was made not to collect stool or serum samples. In the four others (#13, 16–18), the symptom profile suggested bacterial or parasitic disease but laboratory tests on ill persons for bacteria and parasites were negative.

In one of the presumed giardiasis outbreaks (#9), initial reports of numerous laboratory confirmed giardiasis cases dissuaded us from aggressive pursuit of stool specimens only to find that, in fact, physicians' diagnoses were based on clinical picture. In another (#7), all the symptomatic cases had already been treated by the time we were able to collect stool specimens. The only stool positive for *Giardia lamblia* was from a well, untreated camper.

Source of Notification of Outbreaks

Eleven of the 18 waterborne outbreaks were brought to the attention of local health departments by citizens complaining by telephone of illness in themselves and their neighbors; four were brought to state health department attention directly by such citizen phone calls. Three outbreaks were picked up by local health departments in the course of routine active disease surveillance, as clusters of illness were reported by doctors' offices when the local health department made its regular weekly telephone contact. Two outbreaks were reported by physicians, and two outbreaks came to our attention through a combination of direct contacts by ill persons and ongoing review of positive *Giardia lamblia* results in the state laboratory.

No outbreaks were found through 12 months of daily review of positive coliform test results from state water systems although an average of two unsafe coliform results per week were followed up. This activity was therefore discontinued in subsequent years.

Epidemiologic Data

In all 18 outbreaks, a statistically significant association was found between self-reported water consumption and illness (Table 1). In 11 of the 18 outbreaks, a control group on a different water supply was interviewed and had a significantly lower attack rate. Community attack rates ranged from 10 per cent to 64.4 per cent. Outbreaks occurred in all seasons, but 11 of 18 occurred between June 1 and September 30.

Type of Water System

Community water systems** accounted for 15 of the 18 confirmed and seven of the 10 suspected waterborne outbreaks. One confirmed outbreak (#2) was related to a well not intended as a drinking-water supply, and the remainder (two confirmed, three suspected) were on non-community systems. There are 715 community water systems and 1,250 non-community systems registered with the Colorado Department of Health. The largest system with a community-wide problem served approximately 3,500 persons. One outbreak (#1) occurred on a supply serving approximately 50,000 people, but only a few hundred were affected by the cross-connection.

All but one of the outbreaks (#1) occurred on systems using surface water as the source of supply. In three outbreaks (#11, 16, 17), the sources were shallow wells in glacial alluvium immediately adjacent to a river; pollen grains and other particles characteristic of surface water were found when the "well" water was sampled through a one micron filter.

Coliform Counts and Chlorine Residual

Coliform counts were elevated at or near the time of the outbreak in seven outbreaks, normal in nine, and unknown in two. Chlorine residuals were inadequate in six, adequate in eight, and unknown in four outbreaks. In only one of the nine documented or suspected *Giardia lamblia* outbreaks were there elevated coliforms and absent disinfectant at the time of the outbreak. In one Giardia outbreak (#9), coliform counts indicated safe water on two occasions during the outbreak but were elevated two weeks after the outbreak stopped.

Deficiency Responsible for Outbreak

One outbreak (#1) was caused by a contaminated well and another (#2) by a cross-connection. The remaining outbreaks occurred on systems without adequate chemical pretreatment, filtration, and chlorination. In the nine waterborne outbreaks attributed to *Giardia lamblia*, there was either no filtration at all (six outbreaks), a malfunctioning filter and decreased chlorine contract time (one outbreak), or

**Defined as 15 or more service connections and/or 25 or more residents year-round.

TABLE 1—Epidemiologic Features of 18 Waterborne Disease Outbreaks, Colorado, 1980–83

Outbreak No.	Date	Estimated # of cases	Per Cent Attack Rate	Relative Risk[1] (90% C.I.)	Syndrome[2]	Duration	Agent Implicated	Evidence for Agent	Agents Ruled Out[3]	Cause of Outbreak
1.	6/80	15	28.3	4.5 (1.21, 6.7)	C,D,V	1–2 days	unknown	—	—	Cross-connection
2.	7/80	34	70.2	8.46 (3,4,21.06)	D	3–5 days	unknown	—	SS,C	Laundry well used for drinking water
3.	8/80	38	64.4	1.56 (1.13, 2.16)	D,V	2–3 days	unknown	—	SS,C	Chlorinator failure, unfiltered supply
4.	3/81	1500	32.0	2.7 (1.70, 5.38)	D,C,V	3–4 days	Rotavirus	Positive serology & stools in cases clinical picture	SS,C,EEC	Chlorinator failure, filtration inadequate
5.	'81	100	24.0	3.09 (1.52, 6.27)	D,C	3–14 days	*Giardia lamblia*	Positive stools in cases, clinical picture, water filter showed large particles passing through	—	Unfiltered surface source
6.	9/81	600	22.0	2.86 (1.51, 5.4)	D,V	2–3 days	unknown	—	—	Unfiltered surface source
7.	'/81	29	28.0	2.22 (1.17, 4.20)	D	7–10 days	*Giardia lamblia*	Clinical picture in cases, positive water filter	—	Filter off line
8.	11/81	40	18.6	4.21 (1.09, 16.28)	D	7–14 days	*Giardia lamblia*	Positive stools in cases, negative stools in controls, clinical picture, positive filter	SS,C	Shortened chlorine contact time, filter inadequate
9.	11/81	85	29.2	4.0 (1.71, 9.33)	D	4–30 days (mean 8.5)	*Giardia lamblia*	Clinical picture, engineering evaluation	—	Unfiltered pond water backed up into spring box
10.	12/81	135	10.0	8.12 (2.86, 23.03)	D	7–14 days	*Giardia lamblia*	Positive stools	SS	No pretreatment before filtration
11.	1/82	unk	15.1	2.3 (.98, 5.42)	D,C	5–30 days (mean 15.5)	*Giardia lamblia*	Positive stools in cases, clinical picture, positive filter	SS	No pretreatment before filtration
12.	7/82	28	35.0	2.92 (1.32, 6.48)	C,D	7–30 days	*Giardia lamblia*	Stools syndrome	C	Unfiltered surface source, heavy runoff
13.	8/82	600	27.3	3.34 (1.01, 11.02)	D,V	2 days	unknown	—	S,C virus	Unfiltered surface source, heavy runoff
14.	9/82	28	53.8	2.71 (1.59, 4.63)	D,C	5–30 days	*Giardia lamblia*	Stools syndrome, positive filter	unknown	Unfiltered surface source
15.	2/83	50	17.2	6.4 (1.73, 23.65)	D,C,V	3–20 days	*Giardia lamblia*	Stools filter	—	Filtration with inadequate pretreatment
16.	5/83	80	10.0	4.54 (.74, 27.7)	D,C	3–30 days	unknown	—	G,C,SS	Filter overwhelmed by heavy runoff
17.	6/83	300	34.9	2.18 (.66, 7.2)	D,C	1–5 days	unknown	—	G	Filter overwhelmed, heavy runoff, inadequate pretreatment
18.	6/83	370	11.4	6.00 (.71, 51.1)	D,V,C	1–10 days	unknown	—	G,C	No filtration, heavy runoff

1. For persons drinking more than the median amount of water versus those drinking less than the median
2. C = cramps, D = diarrhea, V = vomiting
3. SS = Salmonella/Shigella, C = campylobacter, G = Giardia lamblia, EEC = Enteropathogenic E. coli

filtration without any chemical pretreatment (two outbreaks). In the four suspected or confirmed viral outbreaks, effective filtration was also absent, but the precipitating event was a chlorinator failure (two outbreaks), bypass of a chlorinator (one outbreak), or very high raw water turbidity leading to low free chlorine residuals (one outbreak). Outbreaks #16, 17, and 18 were associated with very high raw and finished water turbidity during spring runoff, when filters were overwhelmed or bypassed.

Specific precipitating events were found for only two of the suspected or confirmed giardiasis outbreaks: backup of beaver pond water into a spring house in #9 decreased chlorine contact time compared to the normal condition in #8.

Discussion

Some of the lessons we have learned from this surveillance experience will be summarized briefly. Daily monitoring and follow-up of coliform tests results failed to uncover any gastroenteritis outbreaks in the communities involved. Increased communication with county health personnel, sanitarians, public health nurses, and physicians apparently paid off, although we were still hampered by delay between the occurrence and reporting of illness. The presence of a single person who reviewed all complaints and all communicable disease reports and initiated investigations was important.

Resort areas may pose special problems in identifying outbreaks. Unsolicited reports from out-of-state visitors alerted us to four of the waterborne outbreaks eventually attributed to *Giardia*. In the Winter Park outbreak (#11), local physicians had seen no increase in diagnosed cases of giardiasis in residents, but letters from visiting skiers convinced us that a problem existed. A survey of out-of-state visitors to the area showed an attack rate of 15.1 per cent for visitors drinking from the town's main water system during the first two weeks of February. Most residents are not served by the town water but most visitors stay in hotels and condominium units served by that water. Long-term residents may have lower attack rates than residents even when comparably exposed.[4]

The decision to classify outbreaks in this study as waterborne was based on demonstrations of a dose-response relationship between water consumption and illness. As long as over- and underestimation of water consumption are not associated with illness status, the magnitude of any observed association between illness and amount of water consumed should be an underestimate.

Ill persons may selectively recall higher water consumption than well persons. Interviewer bias is also possible. In the Eagle-Vail outbreak (#4), however, there was no association between reported water consumption and gastrointestinal illness during the two weeks prior to the outbreak, even though the respondents clearly thought these illnesses were also waterborne.[2] In Aspen Highlands (#8), a relationship was seen between illness and amount of water consumption in short-term but not long-term residents.[4]

Analysis of the data from these two outbreaks using the method described by Marshall *et al.*[5] shows that 30 to 40 per cent of ill persons would have had to overestimate their water consumption by two or more glasses per day (while no well persons overestimated their water consumption) in order to show a dose-response relationship significant at the .05 level when none really existed. Differential misclassification would have to be considerably more common than this to cause a spurious relationship at the levels seen in most of the outbreaks reported here.

The attribution of outbreaks to particular agents was the most difficult and frustrating part of these investigations. Only in one instance, Aspen Highlands,[4] were these major criteria for agent clearly met:

- The agent should be found in ill persons significantly more often than in well persons from the affected community or from an unaffected control community;
- The agent should be found in the epidemiologically incriminated source, the water; or there should be an appropriately timed event that would have allowed the presumed agent to pass through the treatment system.

A positive water sample from a sampling filter is not by itself sufficient evidence that a waterborne outbreak is caused by *Giardia lamblia*. We have been able to find morphologically intact organisms in finished water from communities with no current outbreak.*** Nonetheless we have classified two outbreaks (#7, 9) as presumed giardiasis when there was an appropriate clinical syndrome for giardiasis, evidence of waterborne spread of disease, and evidence by filtration sampling for *Giardia lamblia* in the water, even in the absence of positive stools in ill persons.

The patterns seen in this waterborne disease surveillance effort are consistent with the efficacy of the multiple barrier concept in preventing waterborne disease. None of the 17 surface-water outbreaks occurred on the many systems which have coagulation, flocculation, sedimentation, filtration, and disinfection.

Bacteriological standard violations were associated with only one of the *Giardia* outbreaks (#14). *Giardia lamblia* is relatively resistant to chlorine disinfection. The large number of coliform organisms relative to *Giardia lamblia* in wastes has not allowed the presence of coliforms to predict the presence of *Giardia*.

None of the *Giardia* outbreaks had turbidity violations. Turbidity is a poor measure of the microbiological quality of water in areas (such as Colorado) where raw water turbidities are low. Systems with raw water turbidities below the 1.0 NTU standard often discontinue chemical addition ahead of filtration since it is not needed to meet the standard. Filtration without chemical pretreatment has been shown to be ineffective in removing *Giardia*-sized particles.[6]

Although beaver and other animals have been implicated as *Giardia* carriers, ubiquitous human and domestic animal activity has led us to the conclusion that all surface-water supplies are at risk for *Giardia lamblia*. Since 1977, Colorado has required that all surface water be completely treated, including coagulation, flocculation, sedimentation, filtration, and chlorination steps. This requirement is supported by the absence of reported outbreaks of all types (*Giardia* and viral) where full treatment is employed. Such systems are expensive for small communities. Effective inexpensive surface water treatment methods could substantially reduce the risk of waterborne disease.

***Lapham SC, Hopkins RS, White M, Blair JR, Smades RH, Bissel RC, Simpson GL: Giardiasis in travelers to Colorado. A prospective study of 484 visitors. (Manuscript submitted for publication.)

REFERENCES

1. Water-related Disease Outbreaks. 1982 Surveillance Summary. Atlanta: Centers for Disease Control, August 1983.
2. Hopkins RS, Gaspard GB, Williams FP, Karlin RJ, Cukor G, Bladlow NR: A community waterborne gastroenteritis outbreak: evidence for rotavirus as the agent. Am J Public Health 1984; 74:263–265.
3. Rothman KT, Boice JD: Epidemiologic Analysis with a Programmable Calculator. NIH Pub. No. 79-1649:36–37. Washington, DC: Govt Printing Office, 1979.
4. Istre GR, Dunlop TS, Gaspard GB, Hopkins RS: Waterborne giardiasis at a mountain resort: evidence for acquired immunity. Am J Public Health 1984; 74:602–604.
5. Marshall JR, Priore R, Graham S, Brasure J: On the distortion of risk estimates in multiple exposure level case-control studies. Am J Epidemiol 1981; 113:404–473
6. Logsdon GS, Lippy EC: The role of filtration in preventing waterborne disease. J Am Water Works Assoc 1982; 74:649–655.

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A Three-state Study of Waterborne Disease Surveillance Techniques

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Abstract: For a two-year period, the states of Colorado, Vermont and Washington tested the effectiveness of ten surveillance methods for identifying waterborne disease. Nine were active surveillance methods, soliciting illness reports; one was passive, relying on voluntary disease reporting. One waterborne disease outbreak was identified through use of the nine active methods, while 14 were reported through the passive surveillance method. The presence of coliform bacteria during routine water testing was not related to illness in the community. (*Am J Public Health* 1985; 75:1327–1328.)

Introduction

Since the early 1960s, there has been a steady increase in the reported number of waterborne disease outbreaks in the United States.[1] An average of 41 outbreaks were reported annually, 1979–83. The increased number of reported outbreaks may reflect more complete reporting rather than an actual increased risk.

To examine the methods for improving surveillance of waterborne disease, the US Environmental Protection Agency (EPA) funded three two-year projects by the State Health Departments of Colorado (1980–82), Vermont (1980–82), and Washington (1981–83). Although each project differed somewhat in approach, all were designed to test alternative surveillance techniques and to improve reporting of what would have been previously unreported waterborne disease. Detailed individual reports are available from each state.[2–4]

Methods

Before the implementation of the special surveillance projects, Colorado, Vermont, and Washington detected enteric diseases and disease outbreaks through passive surveillance systems, based upon voluntary reporting with little or no effort devoted to soliciting specific illness reports related to contaminated drinking water. Disease reports from physicians, citizens, and health officials were investigated when there was evidence of an outbreak. Reportable diseases which are caused by potentially waterborne pathogens were reviewed and investigated when drinking water appeared to be a possible source of infection. Salmonellosis and shigellosis are reportable in Colorado, Vermont, and Washington; as of 1984, Colorado and Vermont report giardiasis and campylobacteriosis.

During the project period, each of the three states expanded waterborne disease surveillance activities. A full-time project epidemiologist was employed in each state; Vermont and Washington also employed a part-time project engineer or microbiologist. The project epidemiologists received support from both the state epidemiology and water supply programs for outbreak investigations and water system evaluations. Laboratory support for analysis of medical specimens and water samples was also expanded under the project.

Colorado, Vermont, and Washington applied the waterborne disease surveillance techniques listed in Table 1. Investigations of the water system and user surveys were conducted when any of the surveillance activities revealed contaminated water or excess illness in the community.

Results

The surveillance techniques used by the three state projects identified 68 disease outbreak situations; investigation revealed that only 15 were waterborne. The telephone surveillance of schools, industries, camps, health care providers, and water users identified no waterborne disease outbreaks. The water sampling for bacterial coliforms and for pathogenic bacteria revealed excessive coliforms in some drinking water samples as well as pathogens in raw water supplies. Nevertheless, after investigation, no related illness was identified in the communites served by the water systems.

The collection of epidemiologic data on laboratory diagnosed giardiasis cases in Colorado resulted in the identification of one waterborne giardiasis outbreak. The telephone follow-up of cases of giardiasis, campylobacteriosis, and yersiniosis cases in Washington did not reveal any waterborne disease outbreaks.

Fourteen waterborne disease outbreaks were identified through the enhanced passive surveillance system employed by all three states (10 in Colorado, one in Vermont, and three in Washington). This is the same number of outbreaks identified during the prior two years by the traditional passive surveillance systems of the three states.

Discussion

During the three-state study, the number of reported waterborne disease outbreaks on public drinking water systems doubled in Colorado but decreased in Vermont and Washington. The Colorado results support the national trend of increased waterborne disease outbreaks reported by the Centers for Disease Control; the Vermont and Washington results contradict the national trend.[1] For the three states, the total number of outbreaks increased from 14 for the two prior years to 15 during the project period.

The extent to which these findings can be generalized is unclear. Participating states may have had better than average reporting of waterborne diseases prior to the projects. Willingness to participate in this study is evidence of the interest in waterborne illness. As such, the benefits from increased surveillance may be reduced.

Fourteen of the 15 waterborne disease outbreaks were identified through the traditional passive surveillance method, while one active method, laboratory surveillance of giardiasis cases, yielded one outbreak. Compared to moni-

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TABLE 1—Active Waterborne Disease Surveillance Methods in Colorado, Vermont, and Washington

Method	Time Period (State)	Total Months of Observation
Daily Review of Mandatory Coliform Tests	6/81–5/82 (CO) 9/81–12/81 (VT)	15
Weekly Telephone Surveillance of Schools, Industries, Camps	12/80–9/81 (VT)	9
One-Time Customer Surveys of 12 Water Systems with Water Quality Problems	4/81–3/83 (WA)	—
Twice Weekly Additional Coliform Tests of Selected Water Systems	2/82–12/82 (VT)	10
Pathogen Testing of 60 Water Systems with Water Quality Problems.	4/81–3/83 (WA)	—
Surveillance of Laboratory Confirmed Giardiasis Cases	4/81–3/83 (WA) 6/81–5/82 (CO)	36
Surveillance of Laboratory Confirmed Yersiniosis and Campylobacteriosis Cases	4/81–3/83 (WA)	24
Surveillance of Private Laboratories for Increased Enteric Work Requests	4/81–3/83 (WA)	12
Surveillance of Camps, Parks, Ski Programs, Recreational Organizations	4/81–3/82 (WA)	12
Passive Surveillance Enhanced with Staff Epidemiologist and Public Education	6/80–5/82 (CO) 12/80–12/82 (VT) 4/81–3/83 (WA)	72

toring of laboratory confirmed infections, the passive surveillance system provides the most timely reports. There is an inherent delay in laboratory based surveillance. Stool sample collection, sample delivery to the laboratory, and specimen analysis take one to three weeks, whereas, a physician can become aware of illness in a community and report it within days of the onset.

Under the Safe Drinking Water Act, coliform bacteria is the criterion used to judge the microbiological safety of drinking water. In this study, routine and intensive surveillance of water systems with coliform violations did not reveal illness among water users. Coliform bacteria were elevated in only 10 of the 15 outbreaks which occurred during the project period. The presence of coliforms failed to predict illness in the community. The absence of coliforms did not assure safe drinking water. Although coliform bacteria can be indicative of water contamination, it appears less reliable as an indicator of health risk.

Except in Colorado, the surveillance activities that were undertaken in this study did not result in more reported outbreaks of waterborne disease. The methods used in Vermont and Washington were either too insensitive to pick up additional outbreaks or the current passive surveillance systems employed by the states are adequate. The special surveillance methods described in this paper may be more useful for other local and state public health agencies if current surveillance is inadequate.

REFERENCES

1. CDC: Water related disease outbreaks in the US 1980. MMWR 1982; 30:623.
2. Hopkins RS, Shillam P, Gaspard B, Eisnach L, Karlin RJ: Waterborne disease in Colorado: three years surveillance and 18 waterborne outbreaks. Am J Public Health 1985; 75:254–257.
3. Vogt RL, Little AA: Vermont Waterborne Disease Project. Final Report on EPA contact #68-03-2967. Vermont Department of Health, Burlington, VT 05402.
4. Harter LC, Frost FJ, Perkins-Jones K, Holman R: Washington State Waterborne Disease Surveillance Study. Final Report on EPA Contract #68-03-3059. Washington Department of Social and Health Services. Olympia, WA 98504.

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Routine Coliform Monitoring and Waterborne Disease Outbreaks

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Abstract

To provide protection against the transmission of waterborne disease all public drinking water systems are required to monitor for coliform bacteria at a prescribed frequency. However, waterborne outbreaks have previously been documented in public water systems which have not exceeded these coliform regulations. A comparison of coliform monitoring results in a sample of both community and non-community water systems showed no statistical differences in coliform results between systems in which an outbreak had occurred and systems in which no outbreaks had been reported. Although the results of this study must be interpreted cautiously, the authors feel that public health officials should reevaluate the importance of routine coliform monitoring in their surveillance program for the prevention of waterborne disease.

All public water systems, which include both community systems serving at least 25 year-round residents or 15 service connections and non-community systems serving transients or temporary residents, are required by the U.S. Environmental Protection Agency (EPA) to routinely monitor for coliform bacteria(6). The frequency of coliform monitoring increases as the size of the population served increases, with systems of 1000 persons or less required to collect only one sample per month. It is not until a system serves a population of 25,001 that 30 samples per month are required. There is no requirement that samples be collected daily, and no rationale is provided in the EPA regulations to support the frequency of monitoring.

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The coliform group has been used as an indicator of pollution for some 70 years because they are present in the normal intestinal flora of humans and warm-blooded animals and are eliminated in large numbers in fecal wastes. The absence of coliform organisms in a public water supply is generally interpreted as evidence of a safe drinking water free of waterborne pathogens(6). Implicitly, routine coliform monitoring of drinking water is to provide protection against transmission of waterborne disease.

Outbreaks of waterborne disease have occurred in water systems where the maximum contaminant level (MCL) for coliforms has not exceeded EPA regulations(3,4,5). Case studies have suggested that the current EPA regulations for coliform monitoring frequency, especially for small systems, are inadequate to protect against transmission of waterborne disease and that it is inappropriate to use coliforms for monitoring surface water systems where disinfection is the only treatment(3). Anecdotal information indicates it is also possible to detect coliforms in water systems that have not reported outbreaks.

A study was conducted to compare coliform monitoring results between water systems experiencing a waterborne outbreak and systems in which an outbreak had not been reported and to determine if reported waterborne outbreaks could have been predicted on the basis of violations of the coliform MCL.

The study consisted of two separate but related investigations, one of community water systems and one of non-community water systems. In both cases only the data from existing reports of outbreaks and records of coliform monitoring results were utilized.

Community Systems

Waterborne-disease outbreaks were reported in 47 community water supplies in the United States during the period between 1971-1977. Water supply officials from states in which these outbreaks occurred were contacted, and coliform records for three years (the year preceding, following, and during the time the outbreak occurred) were requested. Records for an identical period were also requested for comparison communities in which an outbreak had not been reported.

Two groups of comparison communities were selected. The first group was selected with the aid of the state water supply officials: a community of about the same size, with the same type of water source, and that employed the same type of water treatment procedures as the outbreak community. To minimize differences in outbreak reporting and water quality surveillance practices (e.g. number of coliform samples collected) between the comparison and outbreak community, the comparison community was selected from the same general geographic area (health district) as the outbreak community. The second group of comparison communities was selected at random from an inventory of water supplies in each county in which an outbreak occurred after excluding systems not within 15% of the same size as the outbreak system. No statistical differences in demographic characteristics were found between the two groups of comparison communities.

These specific questions were examined: (a) Were positive coliform results more likely to occur in the outbreak community during the three months prior to the outbreak? (b) Over the total three-year period, was the coliform standard exceeded more often for those communities experiencing outbreaks? (c) When positive results were obtained over the three-year period, were there more coliform organisms per sample in the outbreak communities?

Unfortunately, much of the data requested was not made available: no monitoring results existed for 10 water systems in communities experiencing an outbreak; four systems had been monitored, but the records had been discarded; for 16 systems the records requested were not made available by the state health officials. Records were provided for 17 community water systems experiencing an outbreak but not all could be used either because the recording methods prevented determination of all required variables or because the records for the comparison community had been discarded. Only 32% of the originally selected data set remained for analysis. Thirteen pairs of communities selected with the aid of state officials and 10 pairs from the random selection procedure had sufficient information for comparison. Regardless of the manner in which the comparison community was selected, there were eight instances where the community experiencing an outbreak and its comparison community had similar coliform results in the three months prior to the outbreak: in four instances both communities had positive coliform results, and in four instances both had negative coliform results.

Because of the small number of pairs of communities with different coliform results, Cox's(2) exact test for discordant pairs was used to evaluate the first question (Table 1). The second and third questions were evaluated by paired comparison procedures using Students' t test (Table 2). The outbreak and comparison communities were not found to differ in regard to positive coliform results within the three months prior to the outbreak (Table 1). It is reasuring, however, that when different coliform results were obtained between the community experiencing an outbreak and its comparison community, the trend was toward detection of coliforms in the outbreak community and negative coliform tests for the comparison community during the three prior months. With respect to the number of times the coliform standard was exceeded per number of samples collected and mean number of coliform organisms in the water system during the entire three year period, no statistical difference was observed between outbreak and comparison communities (Table 2).

Non-Community Systems

In this study, coliform monitoring results for non-community water systems experiencing an outbreak were

Table 1
Community Water Systems
Comparison of Coliform Monitoring Results Three Months Prior to Outbreak

		Comparison Community		
		Positive Test	Negative Test	Total Pairs
A. Comparison Community Selected by State (matched on size, water source, water treatment, location)				
Outbreak Community	Positive Test	4	4	8
	Negative Test	1	4	5
	Total Pairs	5	8	13
	$P_{(1)} = 0.188$			
B. Comparison Community Selected at Random (matched on size, location)				
Outbreak Community	Positive Test	4	2	6
	Negative Test	0	4	4
	Total Pairs	4	6	10
	$P_{(1)} = 0.250$			

Table 2
Community Water Systems
Comparison of Coliform Monitoring Results During 3-Year Period

	Number of Times Standard was Exceeded per Number of Samples During 3-year Period	Mean Coliform Levels Multiplied by Number of Times Standard was Exceeded per Number of Samples During 3-year Period
Outbreak Communities vs. Comparison Communities Selected by States (n = 15 pairs)	$t = 0.27$ $P_{(1)} = 0.395$	$t = 1.07$ $P_{(1)} = 0.151$
Outbreak Communities vs. Comparison Communities Selected at Random (n = 15 pairs)	$t = 1.72$ $P_{(1)} = 0.054$	$t = 1.26$ $P_{(1)} = 0.114$

compared to non-community systems not reporting an outbreak in Pennsylvania during 1973-1978. Pennsylvania was chosen because it reported the largest number of outbreaks during this period, reflecting a dedication to identifying and investigating waterborne disease outbreaks. Thirty-nine waterborne disease outbreaks in non-community systems were reported from Pennsylvania in this six year period.

Data on coliform monitoring of non-community water systems were obtained from county offices. During 1973-1978, non-community water systems in Pennsylvania were inspected at least once a year by a sanitarian and were sampled for coliform analysis if they were considered unsatisfactory with regard to protection and construction or if the disinfection device was not functioning properly. Data were obtained on 34 outbreaks occurring in 18 different counties. There were five instances where two outbreaks of waterborne disease were reported for the same system. The 29 non-community systems with reported outbreaks included 12 restaurants, 12 campgrounds, two recreational areas, one church, one apartment complex, and one fire house. However, no coliform monitoring data were available (apparently no samples had been taken) for 13 of the 29 establishments. This eliminated the church, the apartment complex, the fire house, the two recreational areas, two campgrounds, and six restaurants from the analysis. Coliform monitoring data were also obtained for the remaining 798 non-community water systems for which no waterborne disease outbreaks had been reported in these 18 counties. These included 715 restaurants, 81 campgrounds, and two recreational areas.

The Chi-square test for comparison of two proportions in an independent sample was used to determine if coliforms had been found in a larger proportion of the establishments with reported outbreaks than of the establishments with no reported outbreaks. Discriminant analysis was also employed in an attempt to identify which of several variables were the best predictors of outbreaks of waterborne disease in non-community systems.

This analysis also showed it is possible to find coliforms in non-community systems for which there are no reported outbreaks and to have outbreaks in non-community systems for which there are no positive coliform monitoring results. No difference in positive coliform results was found between non-community systems experiencing an outbreak and those not experiencing an outbreak (Table 3).

For the discriminant analysis, the dependent variable considered was waterborne outbreaks (yes or no) and the independent variables were establishment type, type of water treatment, total number of coliform samples collected over the five year period, and the average coliform density. The only variable showing a statistical association was the total number of coliform samples collected over the five year period, inferring that non-community water supplies with outbreaks are sampled more frequently than those without outbreaks. The frequency of sampling is controlled by a sanitarian who has responsibility for inspecting and sampling non-community water supplies. Apparently, the sanitarians are able to identify by sanitary surveys those water supplies which are most likely to have outbreaks and concentrate the sampling where they think a problem exists. However, the sampling of systems with identified problems did not always cause an outbreak to be avoided. These data provide no evidence that coliform monitoring results provide any prediction of outbreaks of waterborne disease in non-community systems.

Table 3
Non-Community Water Systems
Comparison of Coliform Monitoring Results Three Months Prior to Outbreak

	Coliform Results		
	Positive Result	*Negative* Result	Total
Non-Community System Experiencing an Outbreak	8	8	16
Non-Community System Not Experiencing an Outbreak	343	455	798
Total	351	463	814
$P_{(1)} = 0.288$			

Discussion

These studies suggest that the data obtained from routine coliform monitoring required by EPA regulations may be of little help to public health officials in preventing the transmission of waterborne disease in public water systems; however, these results must be cautiously interpreted for several reasons. The study of community water systems included only 32% of the communities which experienced an outbreak during the study period. This provided a rather small sample which may not be representative of communities experiencing outbreaks. There is also incomplete reporting of waterborne outbreaks and additional outbreaks which were not investigated and reported might have occurred during this period. This would result in unreported outbreaks which were not included in the study, and it is also possible that unreported outbreaks occurred in one or more of the comparison communities. It appears unlikely that comparison communities used in this study experienced a waterborne outbreak that was neither investigated nor reported during the time selected, as outbreak and comparison communities were matched on both geographic location (same local health district) and time to minimize possible differential disease surveillance. The water quality data for both the community and non-community studies were collected from a period before the EPA regulations were applied and may represent incomplete reporting and inadequate monitoring conditions which no longer exist. It is not known to what extent these conditions could influence results, but it is felt that differential monitoring or reporting did not occur. These data also represent results

from a number of different laboratories, but this should not have an influence on interpretation of results, as laboratory procedures are generally standardized for coliform monitoring.

The fact that no differences in coliform monitoring results could be found between the systems with and without reported outbreaks in this study might be explained by several other factors, which were observed during analysis. Of the community systems in the study, the median population was 1,950 and half of the communities in the study were required to sample only two times a month or less. Most non-community systems were sampled only once a year. In instances involving sharp upward fluctuations in contamination immediately prior to the time of outbreak, routine sampling could miss the period of contamination by several days to several months. This situation has previously been documented in several waterborne outbreaks(3). Almost half of the outbreaks in community systems were caused by *Giardia lamblia*. Data from waterborne outbreaks of giardiasis have shown that *Giardia* cysts can be recovered from drinking water meeting the coliform standard, especially for systems providing chlorination as the only treatment, and that waterborne giardiasis outbreaks can occur in water systems meeting the coliform standard(4,5).

There are several possible explanations why the use of routine coliform monitoring may be ineffective in preventing transmission of waterborne diseases. It is possible that the coliform test may neither be sensitive nor specific enough to be useful in alerting officials of the necessity to take action to prevent the occurrence of an outbreak. In addition, routine monitoring may not include enough samples to provide a good probability of detecting the presence of coliforms or an outbreak occurs so quickly after contamination that it is highly unlikely that coliforms would be detected before the outbreak by means other than continuous monitoring. The elapsed time between sample collection and transmittal of results to operating personnel under current regulations, even if contamination is detected, may also be too lengthy to permit corrective action to be taken in a timely manner. A related factor concerns the type of corrective action generally stimulated by a positive coliform result. Too often the initial reaction to a positive result is the collection of a check sample, as it is assumed the result is due to a sampling or laboratory error. Many times check samples continue to be taken until a negative coliform result is obtained, and no positive action is ever taken to determine how the contamination occurred or if there was a treatment or distribution deficiency.

Although the results of this study must be interpreted cautiously and it is recommended that additional studies of this type be conducted, it is, nevertheless, felt that regulatory agencies should carefully examine the relative importance they have assigned to routine coliform monitoring of tap water in their surveillance program. It should be realized that the primary reason for the establishment of such surveillance programs is the prevention of waterborne disease and other kinds of surveillance activities may be more effective and cost efficient in this regard.

The American Water Works Association Committee on the Status of Waterborne Disease in the United States and Canada has just released a report in which they recommend that surveillance activities be structured to help prevent waterborne disease(1). Their conclusions on the usefulness of the coliform test in preventing waterborne disease are of interest:

> "Routine coliform surveillance of treated water is mandated by the Safe Drinking Water Act; however, the committee feels that its importance is to provide a historical record of operation rather than to prevent waterborne outbreaks."

> "The committee feels that it is more important to know the quality of the source water and potential sources of contamination so that source protection and treatment can be provided. A water supply surveillance program should emphasize frequent engineering evaluation and sanitary surveys to identify and correct potential deficiencies. Microbiological resources are better applied to assessing raw water quality, identifying sources of contamination, and evaluating the efficiency of treatment than the routine surveillance of water quality in the distribution system. In some instances, alternative microbiological or chemical indicators, such as chlorine residual analysis, may be more useful in preventing outbreaks than coliform surveillance of the distribution system."

References

1. Committee Report (1981) Waterborne Disease in the United States and Canada, *J. Amer. Water Works Assoc.* 73:528-529.
2. Cox, D.R. (1978) *Analysis of Binary Data,* Methuen and Co. LTD, London, p. 56-58.
3. Craun, G.F. (1978) Impact of the coliform standard on the transmission of disease, *Evaluation of the Microbiology Standards for Drinking Water,* C.W. Hendricks, Ed., USEPA 570/9-78-006, USGPO, Washington, D.C.
4. Craun, G.F. (1979) Waterborne giardiasis in the United States: a review, *Amer. J. Publ. Health* 69:817.
5. Craun, G.F. (1979) Waterborne disease outbreaks in the United States, *J. Environ. Health* 41:259-265.
6. U.S. Environmental Protection Agency (1976) *National Interim Primary Drinking Water Regulations,* USEPA 570/9-76-003, USGPO, Washington, D.C.

AMERICAN JOURNAL OF Public Health reprint

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A Follow-up Study of Gastro-Intestinal Diseases Related to Bacteriologically Substandard Drinking Water

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Abstract: In a prospective follow-up study conducted in 52 French alpine villages, one weekly water sample was taken in each village provided with untreated ground water and analyzed as to the presence of four indicator bacteria: total plate count, total coliforms, thermotolerant (fecal) coliforms, and fecal streptococci. Cases of acute gastro-intestinal disease (AGID) occurring among 29,272 inhabitants were reported through physicians, pharmacists, and primary school teachers. A loglinear model identified fecal streptococcus (FS) as the best predictor; the presence of fecal coliforms enhanced the effect of FS. The total bacteria count and the total coliforms had no independent contributions. A threshold analysis suggested that any level of indicator bacteria above zero was associated with an excess of AGID. (*Am J Public Health* 1987; 77:582–584.)

Introduction

Microbiology standards for drinking water are periodically evaluated.[1] The indicator bacteria used in the European community to assess whether a 100 ml water sample is potable are fecal coliforms (FC) and fecal streptococci (FS).[2] Total coliforms are also used as indicator bacteria in many countries, such as the United States.[3] The dramatic decline of "classical" waterborne diseases in developed countries has lessened the concern of local authorities with these issues. The frequency of substandard water samples is relatively common in the alpine regions, but water-related disease outbreaks are rather rare.[4] Alpine health authorities are highly concerned, however, about the increasing costs and constraints of the water policies.

This study had the following objectives: to assess the risks related to the consumption of drinking water that does not meet current bacteriology standards; to identify the indicator bacteria that best predict this risk; to determine whether there is an indicator bacteria threshold other than zero above which water should be declared "non-potable".

Methods

The study design is presented in detail elsewhere.[5] Two parallel surveys were conducted: 1) a weekly water sample was taken in each of 52 villages and analyzed the same day in a single central laboratory; and 2) a day-to-day count of the acute gastro-intestinal diseases occurring among inhabitants of the study villages and identified through 119 physicians, 52 pharmacists, and 118 primary school teachers (for children aged 7 to 10 years). Physicians and pharmacists were located in the villages or within a 10 km surrounding area. None of these sources was informed of the findings of the weekly water samples.

Villages were chosen to meet several criteria: population between 100 and 3,500; non-tourist areas (to prevent wide variations in village population across seasons); public water systems with untreated ground water; sufficiently far from large cities so that the cases of AGID seeking help could be assumed to be completely enumerated by all the participating professionals.

The limits of the public water system in each village were known to the survey coordinators. Any case reported for an individual not using the municipal water system was excluded from the analysis. The village population was restricted accordingly to users of the public water system.

Although 64 weekly samples were available for most villages, because of technical problems, only 58 samples could be analyzed for some villages. Of the 52 villages available for study, two were excluded prior to examination of the data because of especially large numbers (>14) of missing observations, and two villages were excluded because the ground water they provided was subjected to treatment half-way through the survey, leaving 48 villages in the study group.

The bacteriologic assays conducted to ascertain the presence of indicator bacteria were as follows: 1) "standard plate count" (aerobic bacteria): 1 ml water cultivated on standard medium (PCA Institut Pasteur Production), with a 24-hours incubation at 37° ± 1°C; 2) "total coliforms": 100 ml filtered on cellulose membrane and cultivated on Tergitol TTC during 24 hours at 37° ± 1°C; 3) "fecal coliforms" (thermotolerant coliforms): same technique as for total coliforms but incubated at 42° ± 1°C for 24 hours; 4) "fecal streptococci: 100 ml filtered through cellulose membrane and cultivated 48 hours at 37° ± 1°C on Slanetz and Bartley medium (Institut Pasteur Production).

The 48 villages were followed for 64 weeks and contributed 3,072 "village-weeks". There were 1,807 cases of gastro-intestinal disease reported during the study period. This relatively small number of cases precluded analyzing each village separately across weeks. We assumed that each village-week constituted an independent observation. To avoid being misled by errors in this assumption (i.e., in a given village the water quality was liable to be correlated across weeks), we used a variable that indicated whether cases had occurred the previous week in the same village. This variable was included in the multivariate model to adjust for the experience of the previous week. The unit of observation was the village-week: the cases occurring during a given week in a given village were related to the population of the village (person-week) exposed to the water quality indexed by the weekly sample.

Because of the sparseness of AGID and of the large denominator (each week, 29,272 person-weeks were accrued in the whole set of villages), the events were modeled using a Poisson distribution.

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TABLE 1—Risk of Acute Gastro-intestinal Diseases According to the Fecal Coliforms Exposure, in Absence of Fecal Streptococci, by Village Size

Village Population	Fecal Coliforms	Cases	Person-weeks	Relative Risk* Point Estimate	95% Confidence Interval
<400	Present	3	20 594	.27	[.14; .51]
	Absent	127	237 188		
≥400	Present	17	52 438	1.14	[.67; 1.92]
	Absent	221	777 252		

*RR = rate-ratio

TABLE 2—Relative Risk (and 95% CI) due to Increasing Concentration of Fecal Streptococci, by Level of Fecal Coliforms and Village Size

Village Population	Fecal Coliforms	Fecal Streptococci Count/100 ml 0*	1–5	6–10	11+
<400	Present	1	4.25 (2.1–8.6)	4.74 (1.6–9.6)	5.05 (2.8–9.0)
	Absent	1	1.40 (1.0–2.0)	2.46 (.8–7.7)	2.27 (.8–6.7)
≥400	Present	1	2.72 (1.6–4.1)	1.83 (.9–3.8)	3.21 (1.8–5.6)
	Absent	1	.89 (.7–1.2)	.71 (.3–1.6)	1.44 (.6–3.2)

*Level 0 of FS is the referent category; Relative Risk = 1

Log-linear analysis can be used with Poisson models and accommodate categorical covariates[6–8] yielding estimates of relative risk. Further details on methods and data analysis are discussed elsewhere.* The following covariates were included as potential predictors or confounders of the AGID incidence rate: FS count had four levels (0, 1–5, 6–10, 11 and over, per 100 ml); FC was a binary variable (absent, present); so were total coliforms, total plate count, and village size (under or over 400 inhabitants); and indicator of at least one case the previous week.

The data were restricted to the cases occurring within a three-day period centered on the water sampling day. This restriction, which removed 60 per cent of the total cases from the analysis was implemented to assess and control for a possible misclassification of exposure. Cases far from the sampling day, say the day before the next week or just after the previous week, were liable to result from water pollution as indexed by the water sample of the next week (or the previous week).

The confidence intervals of the relative risks should be computed using the covariances between various pairs of variables. Unfortunately, the Loglin package* used on a Vax 11/780 computer does not generate covariance matrices. Therefore, the confidence intervals of the relative risks presented below were approximated using the appropriate crude (i.e., unadjusted) data tables and the usual formulas for the variance of rate ratios.[9]

Results

The total plate count and total coliforms had no independent predictive value. When the water met the standards (no FC nor FS), the incidence rate was 3.44 per 10,000 person-weeks when at least one germ was observed on the total plate count; it was 3.43 per 10,000 person-weeks when no germ was present. The corresponding incidence rates in the presence of at least one coliform was 3.74 per 10,000 person-weeks, and 3.32 per 10,000 person-week when no coliform was observed.

On the other hand, when both FS and FC were absent, 354 cases occurred, out of a total of 698 (51 per cent). Out of these, 198 cases (53 per cent) occurred when the total plate count was positive, the other half of the cases occurring while no germ was found.

Table 1 presents the raw data for the effect of FC, in absence of FS. This effect is somewhat unclear, and depends on the village size category: in large villages, FC are not related to AGID risk, whereas they seem protective in small villages.

The incidence rates when no FS were found served as baseline risk in further analysis. The absolute risk is rather small; however, it is underestimated by a factor of 2.5 because only the cases occurring within the three-day period about the sampling day were considered. One can infer from Table 2 that the risk due to a given level of FS contamination is higher when FC are also present; the risk due to a given joint FC and FS exposure is higher in small villages than in larger villages; in larger villages, no risk seems associated with FS alone (i.e., when no FC are present), whereas they suffice to predict a significant risk (although weak) in small villages.

These data do not show a clear trend, but it is notable that the highest concentration of FS corresponds to the highest relative risk. The Mantel extension for the analysis of trend[10] was used on the crude data (i.e., unadjusted for the previous week experience) and yielded a significant positive trend only in small villages, in presence of FC [slope = 6.0 $\times 10^{-5}$ cases per person-week per unit of FS increase; χ_1^2 (slope) = 22.6; χ_2^2 = 4.6, testing departure from linearity]. This test for trend was done using the program written by Rothman and Boice for HP.41, applied to incidence rate data.[11]

In almost all strata, the lowest positive level of FS (1 to 5) is associated with a point estimate of the relative risk greater than 1. This class may be too large to assess whether there is a threshold. Another categorization can be adopted, where all concentrations greater than 5 bacteria/100 ml were merged (thus any inference from this highly heterogeneous class would be misleading). Table 3 shows how the relative risk changes with small increases of FS concentration, according to whether some cases had been reported the week before or not. The risk is systematically enhanced when cases had occurred previously. As to the threshold issue, no cut-off point other than 0/1 can be clearly identified.

TABLE 3—Relative Risk (and 95% CI) due to Increasing Concentrations of Fecal Streptococci with Special Focus on Low Levels (adjusting for the previous week experience)

Cases Week Before	Fecal Streptococci Count/100 ml 0	1–2	3–4	5+
None	1	.79 (.6–1.1)	1.56 (1.0–2.4)	1.40 (1.0–1.9)
1+	1	1.47 (1.0–2.1)	2.45 (1.1–5.5)	2.44 (1.8–3.3)

*Berlin J, Zmirou D: Poisson regression for the analysis of relative risk (Unpublished manuscript), Harvard School of Public Health, 1985.

Discussion

The categorization of villages into "small" (<400) and "large" (≥400) populations split the total set of villages into two groups of 24 villages each. Biologic and socioeconomic considerations may contribute to the understanding of the effect of the village size. In small villages, economic activity (agriculture is more oriented toward cattle breeding) and/or general hygiene and wealth may facilitate the spread of a contamination, and make more dramatic the effect of a given waterborne hazard, through direct interpersonal contamination, for example. This hypothesis is consistent with the multiplicative model of risk that underlies the analysis.[9] Although some work has been done on these issues, there is still no unequivocal evidence as to the relationship between indicator bacteria and pathogens, in drinking[12] or non-drinking[13,14] water. In 1981, 44 per cent of the water-related disease outbreaks reported to the Centers for Disease Control (CDC) remained of unknown origin.[15]

This study showed that the four classes of indicator bacteria had very different predictive value with respect to disease. Total bacteria and total coliforms had no predictive value; however, half of the cases of gastro-intestinal disease occurred when the water met the standards. Half of these were observed while only the total plate count was positive.

Those who are primarily interested in identifying which indicator germ best predicts the risk of disease should be concerned with FS and FC (as modifying the effect of FS). Geldreich claims that the FC/FS ratio provides a useful indicator as to the origin of the fecal pollution, human or animal.[16] In our study, the average FC/FS ratio was 4.0 and was different in large villages (5.2) and in small villages (1.3). This tends to confirm that the origin of the contamination in small villages was more related to animals than in larger villages. Those who want to set standards to decide whether a water is safe or not might also be interested in the total plate count. Finally the "background risk" (when all indicator germs are absent) amounts to about one-fourth of total cases; however, this figure may well depend on the specific conditions of this study such as the frequency of the water samples taken in the villages (one per week).

FS was the most predictive indicator and the validity of the standard threshold (0 bacteria per 100 ml) cannot be disputed with these data. We must recognize that our findings depend upon the ecological context and may not hold, for instance, in warm waters or treated waters. Surprisingly, most of the cases of gastro-intestinal disease were sporadic; throughout the 18 months of the survey, only one outbreak was observed (with about 50 cases), even though 42 per cent of the total water samples did not meet the bacteriology standards. Hence, this study shed special light on endemic water-related hazard, which may well be overlooked by health authorities. Although modest, this risk persists, and a lot remains to be done in order to provide a safe and wholesome drinking water to the consumers.

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REFERENCES

1. Hendricks CW (ed). Evaluation of the Microbiology Standards for Drinking Water. USEPA 570/9-78-00C; 1979.
2. Official Journal of the European Communities—No L229/11—Council Directive No 80/778/EEC 15 July 1980 relating to the quality of water intended for human consumption.
3. Federal Register: EPA-Water Programs. National Interim Primary Drinking Water Regulations, 1982.
4. Collin JF, Foliguet JM: A propos de quelques épidémies d'origine hydrique survenues en France. Microbiol Alim Nut 1983; 1:27–33.
5. Ferley JP, Zmirou D, Collin JF, Charrel M: Etude longitudinale des risques liés à la consommation d'eaux non conformes aux normes bactériologiques. Revue Epidém et Santé Publ 1986; 34:89–99.
6. Bishop YMM, Fienberg SE, Holland PW: Discrete Multivariate Analysis: theory and practice. Cambridge: MIT Press, 1975.
7. Laird N,Oliver D: Covariance analysis of censored survival data using log-linear analysis techniques. J Am Stat Assoc 1981; 76:374, 231–240.
8. Frome EL, Checkoway H: Use of Poisson regression models in estimating incidence rates and ratios. Am J Epidemiol 1985; 121:309–323.
9. Kleinbaum DG, Kupper LL, Morgenstern H: Epidemiologic Research. Principles and Quantitative Methods. Lifetime Learning Publications, 1982.
10. Mantel N: Chi-square tests with one degree of freedom; extensions of the Mantel-Haenszel procedure. J Am Stat Assoc 1963; 58:590–700.
11. Rothman KJ, Boice JD: Epidemiologic Analysis with a Programmable Calculator. Boston: Epidemiology Resources, Inc, 1982.
12. Petersen NJ, Hines VD: The relation of summertime gastro-intestinal illness to the sanitary quality of the water supplies in six rocky mountain communities. Am J Hyg 1960; 71:314–320.
13. Robertson WJ, Tobin AS: The relationship between three potential pathogens and pollution indicator organisms in Nova Scotian coastal waters. Can J of Microbiol 1983; 29:1261–1269.
14. Cabelli VJ: Health effects criteria for marine recreational waters, USEPA, 1983, 600/1-80-031.
15. Harris JR, Cohen ML, Lippy EC: Water-related disease outbreaks in the United States in 1981. J Infect Dis 1983; 148:759–762.
16. Geldreich EE: Fecal coliforms and fecal streptococcus density relationship in waste discharges and receiving wastes. Crit Rev Environ Control 1976; 6:349–369.

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Risk Factors for Endemic Giardiasis

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Abstract: In a mail survey, 171 Hitchcock Clinic patients with giardiasis were compared with an age- and sex-matched control group of 684 clinic patients with respect to potential risk factors. Households with shallow well or surface water sources had an odds ratio (OR) for giardiasis of 2.1 (95% confidence interval (95%CI) 1.3-3.2) compared with households with drilled well or municipal water supply. Other observed risks include family member in day care program (OR 2.2, 95%CI 1.3-3.7) and family member with diagnosed giardiasis (OR 17, 95%CI 7.4-37). Previously reported risks such as travel out of country (OR 3.2, 95%CI 1.5-7.2) and camping (OR 1.7, 95%CI 0.9-3.2) were also observed. Virtually no giardiasis risk was observed associated with report of dog or barnyard animal proximity. Control for confounding and adjustment for recall and non-response bias does not materially alter the risk estimates. We suggest that shallow well or surface household water source is an important and previously unrecognized giardiasis risk factor. (*Am J Public Health* 1987; 77:585–587.)

Introduction

Giardiasis is traditionally considered an epidemic disease, often occurring in waterborne community outbreaks.[1-3] Recently, however, increasing numbers of isolated case reports in New Hampshire and Vermont[4,5] suggest multiple sources of endemic infection.

Relatively little has been written about endemic or sporadic giardiasis. Probable risk factors include mountain camping,[6] day care exposure,[7] person to person contact,[8,9] and travel abroad.[10,11] We evaluated these and other potential endemic risk factors such as shallow well household water sources and animal exposure.

Shallow or dug well household water sources were of particular interest to us. Often old and deteriorating, these shallow water sources are frequently open to surface contamination, and are common in northern New England (20 per cent among our controls overall). Several animal species have been implicated as potential giardiasis reservoirs.[12-14] We were interested in the giardia risk conferred by household animal contact and whether such contact interacted with the risk conferred by shallow household water sources.

Methods

We used the Dartmouth-Hitchcock Medical Center laboratory log to identify 190 giardiasis cases seen between January 1, 1977 and June 1, 1984. Nineteen were Dartmouth College students, a highly mobile population, and were not considered in the analysis. The endemic nature of the remaining 171 cases was demonstrated by their failure to cluster by date of diagnosis or zip code; contact with the New Hampshire and Vermont Health Departments confirmed our impression that there had been no epidemic outbreaks of giardiasis in our region during the study period.

Control patients were selected from among the 500,000 Hitchcock Clinic patients in the clinic computer registry (a cumulative data base maintained since 1969). Four age- and sex-matched controls for each case, excluding Dartmouth College students, were randomly drawn from the computer registry. We did not have access to patient medical records, precluding knoweldge of medical diagnosis or vital status.

We mailed a one-page questionnaire to cases and controls asking about place of residence and work, domestic water supply, child day care utilization, animal contacts, foreign travel, household exposure to giardiasis, diarrhea history, and outdoor activities. The questionnaire specified that answers should refer to the year and month of diagnosis for the cases, or the year and month of the case for which a given control was matched. A second mailing was sent to all nonresponders. Finally, we attempted to interview by telephone all the cases and one-third of the controls who did not respond to both mailings (see Table 1).

Standard contingency tables and unconditional logistic regression[15,16] were used to analyze the data. The matching variables (age, sex, year of diagnosis) were included in the logistic models. Recall bias was minimized by also including the year of diagnosis. Response bias was evaluated by weighting the telephone subset to simulate the entire group of non-responding cases and controls in a regression model, and comparing the weighted and unweighted risk estimates. We report exposure frequencies, etiologic fractions,[17] and crude and adjusted odds ratios.[15]

Results

Table 1 summarizes the response patterns for both mailings and the telephone follow-up. The matching criteria are distributed similarly for responding cases and controls (Table 2), while crude exposure rates differ (Table 3).

Table 3 outlines crude and adjusted giardiasis odds ratio (OR) estimates with their 95 per cent confidence intervals (95%CI) and etiologic fractions. Adjustment for matching factors does not materially alter the point estimates. We confirm the influence in our area of previously established risk factors, although the camping risk estimate is imprecise. Shallow well or surface water sources, compared with all other water sources (drilled well or municipal) confers the hypothesized risk for giardiasis (OR 2.1, 95%CI 1.3-3.2).

TABLE 1—Response Rates

	Cases		Controls	
Source	N	%	N	%
Identified	171		684	
1st Mailing	101	59	246	36
2nd Mailing	14	8	75	11
Phone subset	15	9	50	7
TOTAL	130	76	371	54

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TABLE 2—Characteristics of Respondents

Characteristic	Case (130)	Control (371)
Mean Age (years)	31	29
Oldest	76	76
Youngest	2	1
Male	54%	51%
Female	46%	49%
Year of Diagnosis		
1977	14%	14%
1978	8%	10%
1979	9%	14%
1980	13%	12%
1981	15%	15%
1982	15%	15%
1983	21%	16%
1984	5%	4%

"Year of Diagnosis" for Controls refers to that of the matched case.

Compared with municipal water sources, the OR estimate is even larger (OR 2.6, 95%CI 1.6-4.3). Adjustment for matching factors, travel history, age of well, or animal exposure does not substantially change the estimate. Animal exposure is not clearly associated with disease, with the possible exception of cats. Similar results are found when the analysis is limited to households with shallow wells.

Discussion

This case-control study of non-epidemic giardiasis identifies several risk factors of importance in rural northern New England. We confirm the importance of some established risk factors (foreign travel, day care center exposure, and household case contact) and calculate a positive but unstable risk estimate for camping (Table 3). We also find a previously unrecognized giardiasis risk for the household use of shallow water sources, while domestic and barnyard animal contact does not seem to be important.

Shallow wells are a common household water source in northern New England, and their association with giardiasis may be an important public health consideration. Inadequate construction or maintenance may be responsible for surface or septic contamination. Among households with shallow wells, those with pets or livestock did not have an increased giardiasis risk, perhaps reflecting a lack of importance of domestic animal reservoirs.[13,14] We believe our questionnaire did not reliably ascertain exposure to wild animals since the reported prevalence was unexpectedly low for this region. Risk estimates for wild animal proximity are extremely imprecise and are excluded from this report.

The risk associated with household contact may operate through contaminated water, but this may simply represent person to person contact or common exposure to a different risk factor. Large urban day care centers are recognized as an important source of giardiasis[7]; we observed increased risk among those exposed to children attending small rural day care centers. Camping and domestic animal exposure in northern New England appear to be less important giardiasis risk factors than in other parts of the United States.[6,12] The etiologic fraction (14 per cent) for cat exposure incorporates an unstable odds ratio estimate and should be interpreted with caution.

Several potential biases are possible in this retrospective case-control study. Recall bias was evaluated using logistic models that included year of diagnosis as a covariate. This adjustment did not materially affect the risk estimates. The Hitchcock Clinic records from which the controls were selected included patients seen since 1969, although cases occurred only since 1977. To evaluate the potential impact of this difference in time frames, the analysis was repeated using the subset of controls with patient billing activity since 1980 (a list through 1977 was unavailable). This subset analysis generated risk estimates similar to those reported for the entire sample.

Response bias was controlled by weighted logistic regression in which the cases and controls interviewed by telephone were weighted to represent all non-respondents to the mailings. Crude and weighted odds ratios appear in Table 4. The point estimates differ substantially only for "travel abroad" and "household contact", but these are based on small numbers of exposed cases and are therefore unstable. Overall, we do not think nonresponse greatly distorted our findings. Detection bias was possible if physicians tested for giardiasis more commonly among returning travelers or household contacts of known cases. We believe this may have inflated our risk estimates for these exposures.

Information bias may misclassify water source information. We did not ask if municipal water sources were filtered—a water treatment known to reduce water supply giardiasis risk. However, in 1981 fewer than 20 per cent of the community water sources in our region were filtered. Since we do not know the addresses of controls at the time of case diagnosis, we are unable to establish the filtering status of municipal water sources in our data. However, failure to control for this would tend to bias conservatively our estimate of the giardiasis risk associated with shallow wells. Our information regarding onsite water sources is probably ac-

TABLE 3—Risk Measures for Giardiasis

Risk Factor	% Exposure		% Etiologic Fraction	Crude OR		Adjusted OR*	
	Case	Control		OR	95% CI	OR	95% CI
Foreign Travel	11	4	8	3.3	(1.5–7.2)	4.2	(1.8–9.5)
Camping	14	8	5	1.7	(0.9–3.2)	1.6	(0.6–3.1)
Day Care	23	12	12	2.2	(1.3–3.7)	2.2	(1.2–4.0)
Household Case	31	12	29	16.5	(7.4–37)	21.0	(8.8–50)
Shallow Well	34	20	18	2.1	(1.3–3.2)	2.1	(1.3–3.2)
Household Dog	56	57	0	1.0	(0.7–1.5)	0.9	(0.6–1.4)
Household Cat	48	41	14	1.4	(0.9–2.0)	1.3	(0.9–2.0)
Farm Animals	14	12	2	1.2	(0.7–2.2)	1.1	(0.6–2.1)

*Adjusted for matching factors: age, sex, year of diagnosis.

TABLE 4—Impact of Potential Response Bias

Factor	Mailing OR	Weighted OR
Shallow Well	2.2	1.9
Day Care	2.2	2.1
Travel	3.0	5.4
Household Contact	15	27

curate: rural homeowners are aware of household water source, since municipal or drilled water sources can substantially improve the market value of a home.

It is possible that some of our controls may have had giardiasis. Among controls, 3 per cent indicated a past diagnosis of the condition, while 7 per cent reported a prolonged episode of diarrhea. Because we could not verify the giardiasis status of controls, we did not exclude respondents on the basis of self-reported disease. This misclassification would tend to bias observed risks toward the null; we believe this conservative approach justifiable in the absence of systematic disease ascertainment on all controls.

We found no evidence of important confounding by the matching variables (age, sex, and year of diagnosis) using unconditional logistic regression (Table 3). Adjustment for camping and foreign travel did not alter the risk estimates for shallow well household water source. We cannot exclude the possibility of residual confounding by unknown factors. In particular, socioeconomic factors that may influence the type of water source might also influence other giardiasis risks.

In summary, our study confirms the previously reported endemic or sporadic giardiasis risks of foreign travel, day care exposure, and household case contacts. We suggest that shallow well household water sources are an important and previously unreported risk for endemic giardiasis in northern New England. The 18 per cent etiologic fraction attributable to this exposure suggests that it has a significant impact on endemic giardiasis.

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REFERENCES

1. Shaw PK, Brodsky RE, Lyman EO, *et al*: A community wide outbreak of Giardisis with evidence of transmission by a municipal water supply. Ann Intern Med 1977; 87:426–432.
2. Lippy EC: Tracing a giardiasis outbreak at Berlin, New Hampshire. J Am Water Works Assoc 1978; 70:512–520.
3. Lopez CE, Dykes AC, Juranek DD, Sinclair SP, Conn JM, Christie RW, Lippy EC, Schultz MG, Mires MH: Waterborne Giardiasis: A communitywide outbreak of disease and a high rate of asymptomatic infection. Am J Epidemiol 1980; 112-495–507
4. Vermont Department of Health, Epidemiology Division: Vermont Disease Control Bulletin 1985; 2.
5. New Hampshire Division of Public Health Services: Giardiasis Study Planned. Communicable Disease Rep Lab News 1983.
6. Wright RA, Spencer HC, Brodsky RE, Vernon TM: Giardiasis in Colorado: An epidemiologic study. Am J Epidemiol 1977; 105:330–336.
7. Pickering LK, Woodward WE, DuPont HL, Sullivan P: Occurrence of Giardia lamblia in children in day care centers. J Pediatr 1984; 104:522–526.
8. Meyer EA, Jarroll EL: Reviews and commentary: Giardiasis. Am J Epidemiol 1980; 111:1–12.
9. Schmerin NJ, Jones TC, Klein IJ: Giardiasis associated with homosexuality. Ann Intern Med 1978; 88:801–803.
10. Fiumara N: Giardiasis in travelers to the Soviet Union. N Engl J Med 1973; 288:1410–1411.
11. Brodsky RE, Spencer HC, Schultz MG: Giardiasis in American travelers to the Soviet Union. J Infect Dis 1974; 130:319–323.
12. Davies RB, Hibler CF: Animal reservoirs and cross-species transmission of Giardia. *In*: Jakubowski W, and Hoff JC: EPA Symposium on Waterborne Transmission of Giardiasis. EPA Pub. No. 600.9-79-001. Cincinnati: EPA, 1979.
13. Kirkpatrick C, Green G: Susceptibility of domestic cats to infections with Giardia lamlia cysts from human sources. J Clin Microbiol 1985; 21:678–679.
14. Hewlett E, Andrews J, Ruffier J, *et al*: Experimental infection of mongrel dogs with giarrdia lamblia cysts and trophozoites. J Infect Dis 1982; 145:89–93.
15. Breslow N, Day N: Statistical Methods in Cancer Research: Vol 1-The Analysis of Case-Control Studies. Lyon: Internationl Agency for Research on Cancer, 1980.
16. SAS Institute: SAS User's Guide. Cary, NC: SAS Institute, 1982.
17. Kleinbaum D, Kupper L, Morgenstern H: Epidemiologic Research. Belmont, CA: Lifetime Learning Publications, 1982.

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EPIDEMIOLOGIC SURVEILLANCE FOR ENDEMIC *GIARDIA LAMBLIA* INFECTION IN VERMONT

THE ROLES OF WATERBORNE AND PERSON-TO-PERSON TRANSMISSION

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Birkhead, G., and R. L. Vogt (Vermont Dept. of Health, Burlington, VT 05402). Epidemiologic surveillance for endemic *Giardia lamblia* infection in Vermont: the roles of waterborne and person-to-person transmission. *Am J Epidemiol* 1989;129:762–8.

The authors studied 1,211 laboratory-confirmed, non-outbreak-related cases of giardiasis in Vermont residents reported through Vermont's laboratory-based, active surveillance system between 1983 and 1986. Giardiasis was the most common reportable disease in the state, with an average annual incidence rate of 45.9 cases per 100,000 population per year. This rate is higher than that in other states reporting giardiasis incidence. Morbidity from giardiasis was also significant in that 30% of cases reported symptoms lasting four or more weeks. Waterborne transmission was suggested to be an important cause of non-outbreak-related cases because rates of infection were highest in persons receiving nonfiltered municipal or nonmunicipal residential drinking water. Rates were also higher at higher elevations, where water supplies may be difficult to protect from contamination. In addition, the pattern of age-specific incidence rates and the high estimated incidence of infection in children attending day care suggested that person-to-person transmission also played a role in causing non-outbreak-related cases. Routine surveillance data can serve to indicate likely important routes of transmission of giardiasis in the community.

***Giardia*; giardiasis; water supply**

Giardia lamblia has been recognized as a human pathogen since outbreaks of giardiasis were first described in the 1960s and 1970s (1, 2). It is the most commonly determined cause of waterborne disease epidemics in the United States in terms of both the number of outbreaks and the number of persons affected (3). In addition, there have been numerous reports of the high prevalence of giardiasis in child day-care settings due to person-to-person transmission (4). However, the extent of endemic, or non-outbreak-related, giardiasis and the risk factors for endemic transmission in the United States are less well described, partly because this infection has only recently been made reportable in many states and few population-based data are available.

Epidemiologic surveillance systems are one source of information used to determine the predominant patterns of disease transmission in a community. Giardiasis

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has been subject to epidemiologic surveillance in Vermont since it was made a reportable disease on July 1, 1979. We report on a review of Vermont giardiasis surveillance data for 1983 to 1986 conducted to better define the epidemiology of endemic giardiasis in the state.

MATERIALS AND METHODS

The Vermont Department of Health conducts active laboratory surveillance for reportable diseases and has previously reported on the attributes of this system (5, 6). Briefly, all 18 licensed clinical laboratories in the state are required to contact the Epidemiology Division weekly by telephone or by mail whether or not they have cases to report. Laboratories failing to report regularly are contacted by the health department. Physicians are also required to report cases.

A public health nurse interviews each case using a standard form recording demographic information, the date of onset and nature of symptoms, and possible risk factors for infection. The risk factors inquired about are residential drinking water source, child day-care attendance, drinking of untreated water while away from home (e.g., while hiking), and history of foreign travel in the month before illness. The nurse offers free laboratory stool testing to asymptomatic members of case households and recommends that those with symptoms be treated by a physician.

For this study, a case of giardiasis was defined as a Vermont resident with *G. lamblia* cysts or trophozoites in the stool who either had onset of symptoms of giardiasis (diarrhea or cramping) beginning in the period January 1, 1983 through December 31, 1986, or, if asymptomatic, submitted the laboratory specimen during this time. Laboratory reports were examined through March 31, 1987, to ensure complete ascertainment of 1986 cases. Reports were not included in the study if the case was part of a recognized community outbreak or child day-care center outbreak (two or more cases with the same probable source of information and not living in the same household), was a recently arrived refugee, or had previously reported giardiasis. Symptom and risk factor information was reviewed in detail for 1985 cases.

Only symptomatic cases were included in incidence calculations because we assumed that a larger and more constant proportion of symptomatic than asymptomatic cases come to medical attention. We felt that information on symptomatic cases would then be more representative of all symptomatic cases and less subject to biases related to ease of attaining medical care or other factors. In addition, the date of infection for incidence calculations could be known with certainty only for symptomatic cases. Finally, including asymptomatic cases identified in the case-household investigations could bias incidence rates downward in groups less completely screened, such as adults (compared with children) or persons in areas of the state served by a less vigorous public health nurse.

Age- and sex-specific population estimates for Vermont for 1983–1986 were used to calculate rates (7). Incidence rates by town were calculated from the median number of cases per town during the four-year period.

The rates of symptomatic *G. lamblia* infection were calculated for persons with different sources of residential drinking water. For this analysis, cases were excluded if they reported other possible risk factors for infection such as day-care attendance, contact with a day-care attendee, drinking of untreated water while away from home, or a history of recent foreign travel. Cases with a household member who had *G. lamblia* infection beginning more than three days before their own illness were also excluded to eliminate cases that were possibly due to person-to-person transmission in the home. Residential water sources were categorized as municipal or private. Municipal systems were further categorized by type of source (surface

or well) and by type of treatment for surface sources (filtered or not filtered). The types of water systems serving each town and estimates of the population served in 1985 were obtained from the municipal water system inventory of the Environmental Health Division, Vermont Department of Health. Since we could not determine the type of water system supplying the homes of giardiasis cases in towns with multiple municipal water systems, we assumed that cases were on the system serving the largest population in the town. We calculated the total population receiving drinking water from nonmunicipal water systems by subtracting the number of persons on municipal systems from the total state population. We determined the risk of *G. lamblia* infection by elevation of town of residence using each town's elevation above sea level (8).

The rate of infection was also calculated for children attending day care. An estimate of the number of children under age five years attending licensed or registered day-care centers in the state in 1985 was obtained from the Department of Social and Rehabilitative Services, State of Vermont.

RESULTS

A total of 1,406 laboratory-confirmed cases of giardiasis in Vermont residents were reported to the Vermont Department of Health between 1983 and 1986 (table 1). Of these, 1,211 (86 per cent) cases were not outbreak-related. These form the basis of the study. Case investigation forms were completed for 1,202 (99 per cent) cases. Symptomatic cases accounted for 977 (81 per cent) and asymptomatic cases for 234 (19 per cent) of the cases. A total of 127 outbreak-related cases were identified as being part of one community outbreak or more than 20 day-care outbreaks. Refugees, predominantly from southeast Asia, accounted for the remainder of the cases.

The mean annual incidence rate for symptomatic cases was 45.9 cases per 100,000 population per year (table 1). Giardiasis was the most common reportable enteric disease in Vermont during this time. The occurrence of symptomatic cases peaked during the months of July through November (figure 1). This pattern was evident regardless of residential drinking water source or whether drinking of untreated water outside the home was reported (data not shown). The rate of symptomatic *G. lamblia* seemed to be highest in towns located in the mountainous northern and central regions of the state (figure 2).

The mean annual age- and sex-specific incidence rates for symptomatic cases for 1983 to 1986 are shown in figure 3. The highest rates are in children aged one to four years. In this age group, males had 1.5 times the rate of symptomatic giardiasis of females (95 per cent confidence interval (CI) 1.1–2.0). The rate was also high for persons, especially women, aged 20 to 39

TABLE 1

Laboratory-confirmed cases of giardiasis reported to the Vermont Department of Health, 1983–1986: Vermont Department of Health giardiasis surveillance data

Year	Non-outbreak-related			Outbreak-related		Refugees	Total reported cases
	Symptomatic cases	Rate/100,000 population*	Asymptomatic cases	Community outbreak	Day-care outbreak		
1983	163	31.0	39	0	22	26	250
1984	306	57.7	73	0	66	26	471
1985	252	47.1	57	0	9	7	325
1986	256	47.4	65	24	6	9	360
Total	977	45.9	234	24	103	68	1,406

* Population estimates by the Vermont Department of Health (7).

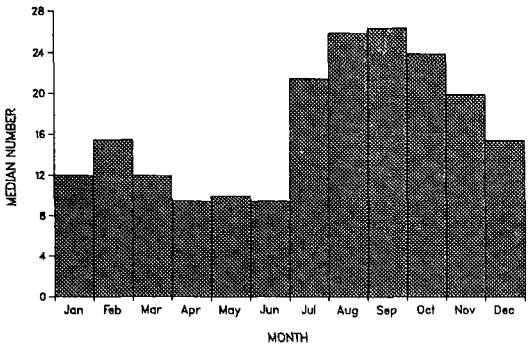

FIGURE 1. Number of symptomatic *G. lamblia* infections, by month, in Vermont, 1983–1986. Vermont Department of Health giardiasis surveillance data.

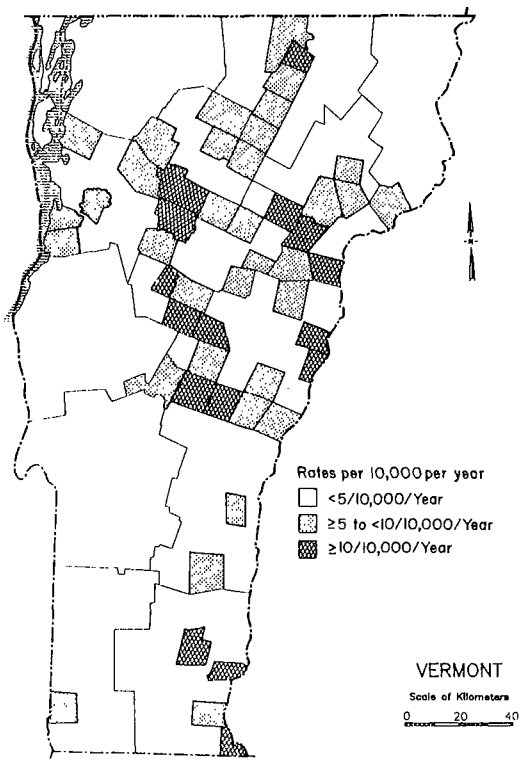

FIGURE 2. Median yearly incidence rate of symptomatic *G. lamblia* infection, by town, in Vermont, 1983–1986. Rates are given per 10,000 population per year. Vermont Department of Health giardiasis surveillance data.

years. Women in this age group were 40 per cent more likely to have symptomatic giardiasis than men (relative risk (RR) = 1.4, 95 per cent CI 1.1–1.6).

Diarrhea was the most commonly reported symptom (96 per cent), followed by cramps (64 per cent) and weight loss (48 per cent). Thirty per cent of symptomatic cases reported having symptoms lasting more than four weeks. The most commonly reported risk factor for infection was history of contact with a person with diarrhea in the last month (38 per cent), followed by drinking of untreated water while away from home (13 per cent), attendance at day care (12 per cent), contact with a day-care attendee (8 per cent), and foreign travel (6 per cent).

Information on the source of residential water supply was available for 243 (96 per cent) symptomatic 1985 cases, of whom 118 received their drinking water from municipal water systems and 125 obtained it from nonmunicipal water sources. The health department's water systems inventory showed that an estimated 56 per cent (300,273) of the state's population was served by municipal water systems in 1985. Using these data, we estimated that state residents with nonmunicipal residential water systems have approximately a 30 per cent excess risk of developing symptomatic giardiasis compared with those on municipal water systems (RR = 1.3, 95 per cent CI 1.0–1.7). The rates of giardiasis varied by type of water system (table 2). Nonmunicipal water system users had 2.2 times the rate of giardiasis of those on filtered, surface water municipal systems. Municipal surface water systems without filtration had almost twice the rate of giardiasis of those with filtration. Rates were elevated for users of municipal well water systems. The risk of infection also increased with increasing elevation of town of residence (table 2).

Among 92 cases less than five years of age, 36 (39 per cent) attended day care. The Department of Social and Rehabilitative Services estimated that 12,000 positions were available in licensed or registered day-care centers in 1985, which meant that 29.4 per cent of children in the state under age five years were in officially sanctioned day

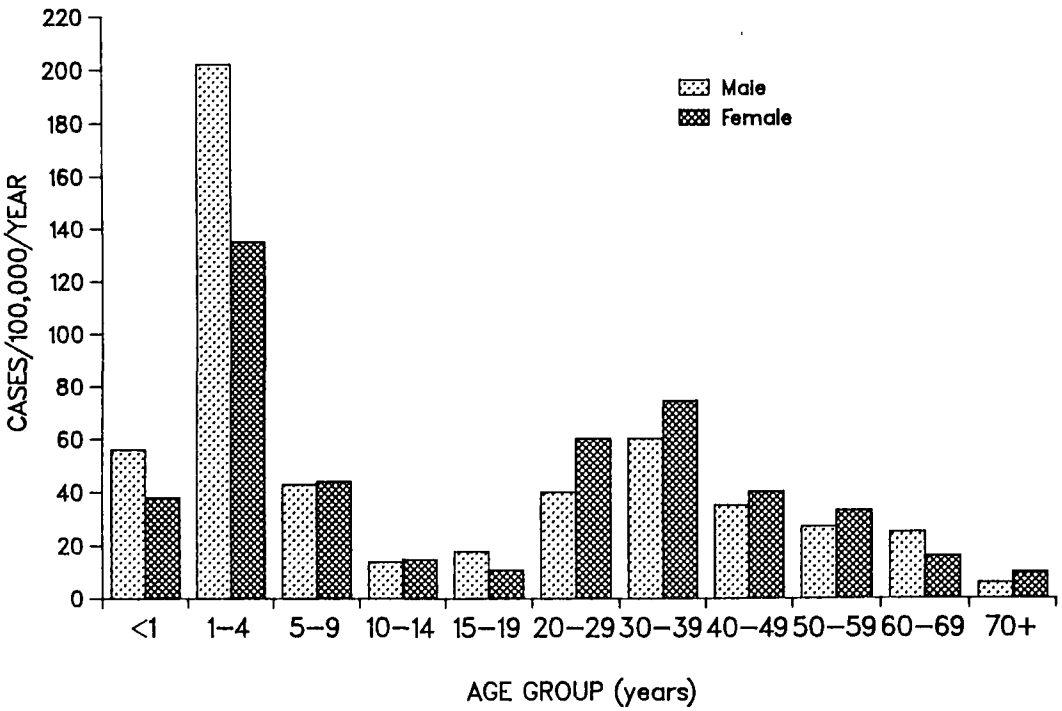

FIGURE 3. Incidence rates of symptomatic *G. lamblia* infection, by age and by sex, in Vermont, 1983–1986. Vermont Department of Health giardiasis surveillance data.

TABLE 2

Risk factors for development of G. lamblia infection in Vermont, 1985: Vermont Department of Health giardiasis surveillance data

Risk factor	*n*	Population at risk	Rate/100,000 population	RR*	95% CI*
Residential water supply†					
Municipal					
Surface water, filtered	20	132,178	15.1	1.0	
Surface water, not filtered	30	104,732	28.6	1.9	1.1–3.3
Well water	17	63,363	26.8	1.8	0.9–3.4
Private	77	234,727	32.8	2.2	1.3–3.6
Total	144	535,000			
Child day-care attendance‡					
Attendee	36	12,000	300.0	1.0	
Nonattendee	56	28,762	194.7	1.5	1.0–2.3
Total	92	40,762			
Elevation of town of residence (m)§					
0–99	190	135,817	35.0	1.0	
100–199	394	222,399	44.3	1.3	1.1–1.5
200+	389	173,598	56.0	1.6	1.4–1.9
Total	973	531,814			

* RR, relative risk; CI, confidence interval.

† Cases without other risk factors for giardiasis.

‡ Cases aged less than five years.

§ Case data for 1983–1986, missing elevation for five towns and missing town of residence for four cases. Rates of *G. lamblia* infection in this section are calculated per 100,000 population per year.

care, assuming full enrollment. The relative risk of day-care attendees compared with that of nonattendees is shown in table 2.

Discussion

Our review of surveillance data showed that *G. lamblia* was the most common reportable enteric disease in Vermont during the period 1983–1986. The incidence rate of symptomatic giardiasis (45.7 per 100,000 population) in Vermont is much greater than rates reported previously by other states. In Minnesota, the incidence rate was 9.8 per 100,000 population based on data from the state laboratory for 1971 through 1975 (9). A survey of most laboratories in Colorado in 1972 and 1973 found a giardiasis incidence rate of 11.6 per 100,000 population per year (10). This had increased by 1981–1982 to approximately 16 per 100,000 population per year based on state laboratory reports and active case finding among most primary-care providers (11). These reports did not exclude asymptomatic, outbreak-related cases or cases in refugees.

One explanation for the higher giardiasis incidence rate in Vermont is that the active, laboratory-based reporting and active case investigations may detect a greater proportion of cases than do other state surveillance systems. In addition, physician recognition and diagnosis of giardiasis may have improved since the previous reports. Finally, the incidence of giardiasis may truly be higher in Vermont than in other states, perhaps partly because of the rural nature of Vermont and the possibly higher frequency of contact with sources of infection in the environment, particularly water. The true giardiasis incidence in Vermont is probably higher than that reported here because some state residents may leave the state to be diagnosed. This is a threat to the sensitivity of most surveillance systems. We have no information on the number of these cases, but we suspect that they are probably few.

Our data suggest that waterborne transmission is an important source of nonoutbreak-related *G. lamblia* infection in Vermont. Persons receiving drinking water from nonmunicipal residential drinking water systems, which often have an unprotected water source and may not be filtered, had the highest rate of symptomatic giardiasis of any group. Municipal water systems with filtration had the lowest rates of giardiasis, which is in accord with the knowledge that filtration is necessary for adequate treatment of water to remove *Giardia* cysts (3, 12). Shallow, poorly constructed wells have been shown by others to be a risk factor for giardiasis (13). The increased risk of infection among users of municipal well water in Vermont suggests that some of these systems are not adequate to prevent giardiasis.

The increase in the rate of infection with elevation also suggests that waterborne transmission is occurring. Outbreaks of giardiasis in mountainous areas have been traced to drinking contaminated water (14). Rates of illness are higher in these areas where easily contaminated surface waters may be the predominant source of drinking water and where low water temperature may prolong the survival of *Giardia* cysts (14). A final point in favor of the possibility that waterborne transmission is important in endemic giardiasis is that more cases had onset in the summer months when outdoor activities bring more people into contact with possibly contaminated water. It is unclear why the peak in the number of cases continued into the fall.

Our data are limited by lack of knowledge of the exact municipal water source for cases in towns with multiple water systems and by the crude nature of the estimates of the population served. It is possible that other factors associated with having unfiltered municipal or nonmunicipal residential drinking water, such as lower socioeconomic status, poorer access to medical care, or crowding, may have a role in the increased risk of infection in this group. The variable sensitivity of the giardiasis surveillance system in different regions of the state may also contribute to some of the

differences in rates. These factors are unlikely to significantly affect our results because of the consistency of relative risks associated with different types of water supply and elevation and because the more rural parts of the state, where access to medical care and reporting might be expected to be the poorest, had the highest rates of giardiasis.

Our data also indicate that person-to-person transmission may play a role in non-outbreak-related giardiasis. Children aged one to four years had the highest rates of symptomatic infection of any age group. This pattern has been described in other enteric infections (15–18) and may be due to poorly developed immunity or increased fecal-oral contact among children. Child day-care centers are a known site of person-to-person transmission among children (4). The higher rates observed for male children are unexplained, but a similar pattern has been observed by others with both *Giardia* and *Campylobacter* infections (18).

The data we have presented indicate that *G. lamblia* may be a more common pathogen than has been previously recognized from reports of outbreak investigations. Surveillance data are limited in identifying causative factors by their descriptive nature, but where data are available on the populations at risk, they can serve to confirm known risk factors and to estimate their importance in disease transmission in the community.

References

1. Moore GT, Cross WM, McGuire D, et al. Epidemic giardiasis at a ski resort. N Engl J Med 1969;281:402–7.
2. Shaw PK, Brodsky RE, Lyman DO, et al. A community-wide outbreak of giardiasis with evidence of transmission by a municipal water supply. Ann Intern Med 1977;87:426–32.
3. Centers for Disease Control. Water-related disease outbreaks: annual summary, 1983. Atlanta, GA: Centers for Disease Control, September 1984.
4. Pickering LK, Woodward WE, DuPont HL, et al. Occurrence of *Giardia lamblia* in children in day-care centers. J Pediatr 1984;104:522–6.
5. Vogt RL, Clark SW, Kappel S. Evaluation of the state surveillance system using hospital discharge diagnoses, 1982–1983. Am J Epidemiol 1986;123: 197–8.
6. Harter L, Frost F, Vogt RL, et al. A three-state study of waterborne disease surveillance techniques. Am J Public Health 1985;75:1327–8.
7. Vermont Department of Health. 1985 estimates of the population and housing of Vermont counties and towns. (Vermont public health statistics bulletin). Burlington, VT: Vermont Department of Health, March 1986.
8. Hennenberger JD, ed. 1982 Vermont year book. Chester, VT: The National Survey, 1982:135–507.
9. Weiss HB, Winegar DA, Levy BS, et al. Giardiasis in Minnesota 1971–1975. Minn Med 1977;60:815–20.
10. Wright RA, Spencer HC, Brodsky RE, et al. Giardiasis in Colorado: an epidemiologic study. Am J Epidemiol 1977;105:330–6.
11. Hopkins RS, Shillam P, Gaspard B, et al. Waterborne disease in Colorado: three years surveillance and 18 outbreaks. Am J Public Health 1985; 75:254–7.
12. Logsdon GS, Symons JM, Hoye LH. Water filtration techniques for removal of cysts and cyst models. In: Jakubowski W, Hoff JC, eds. Waterborne transmission of giardiasis. Cincinnati, OH: US Environmental Protection Agency, 1979:240–56. (Publication no. EPA-600 9-79-001).
13. Chute CG, Smith RP, Baron JA. Risk factors for endemic giardiasis. Am J Public Health 1987; 77:585–7.
14. Meyer EA, Jarroll EL. Giardiasis. Am J Epidemiol 1980;111:1–12.
15. Ryder RW, Merson MH, Gangarosa EJ. Salmonellosis in the United States, 1968–1974. J Infect Dis 1976;133:483–6.
16. Blaser MJ, Pollard RA, Feldman RA. *Shigella* infections in the United States, 1974–1980. J Infect Dis 1983;147:771–5.
17. Tauxe RV, Pegues DA, Hargrett-Bean N. *Campylobacter* infections: the emerging national pattern. Am J Public Health 1987;77:1219–21.
18. Hopkins RS, Olmstead RN. *Campylobacter jejuni* infections in Colorado: unexplained excess of cases in males. Public Health Rep 1985;100: 333–6.

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Cryptosporidiosis and Surface Water

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Abstract: In the period July through October, 1986, 78 laboratory-confirmed cases of cryptosporidiosis were identified in New Mexico. To determine possible risk factors for development of this disease, we conducted a case-control study; 24 case-patients and 46 neighborhood controls were interviewed. Seventeen (71 per cent) of the 24 case-patients were females, seven (29%) were males; their ages ranged from 4 months to 44 years, median 3 years. There was a strong association between drinking surface water and illness: five of the 24 case-patients, but none of the 46 controls drank untreated surface water. Among children, illness was also associated with attending a day care center where other children were ill (odds ratio = 13.1). (*Am J Public Health* 1989; 79:39–42.)

Introduction

Cryptosporidium, first recognized as a cause of diarrhea in humans in 1976,[1] has become known as a common cause of diarrhea worldwide.[2,3] No effective therapy is known; the diarrhea is often prolonged and accompanied by weight loss. In immunocompromised persons, cryptosporidiosis can cause a life-threatening dehydration.

Cryptosporidium can be transmitted through contact with animals, especially cattle and sheep,[4] through person-to-person contact, especially in day care centers,[5] and through a contaminated community water source.[6] In several reported outbreaks, the source of the infection was unknown.[7,8]

In 1984, 24 cases of cryptosporidiosis were reported to the New Mexico Office of Epidemiology, 13 of them were associated with two day care centers. In 1985, 24 cases were reported. In July 1986, a cluster of 20 cases of cryptosporidiosis was recognized in Bernalillo County (the city of Albuquerque). Because these cases did not appear to be related and the source of infection was unclear, a case-control study to evaluate potential risk factors for illness was conducted.

Methods

Since February 1984, all stool samples submitted to the New Mexico Health and Environment Department's Scientific Laboratory Division for ova and parasite testing have been examined for *Cryptosporidium*. Stool samples are concentrated using a formalin-ethyl acetate technique. A heat-fixed fecal smear is stained with Kinyoun modified acid-fast stain[9] and counterstained with Loeffler methylene blue. The specimen is then examined under a light microscope for the presence of *Cryptosporidium* oocysts.

In August 1986, the Office of Epidemiology sent a letter to primary care physicians in Bernalillo County, alerting them to the cluster of cases, reviewing the symptoms and diagnosis of cryptosporidiosis, and requesting that stool samples be sent if cryptosporidiosis was suspected.

A case-patient was defined as a Bernalillo County resident with laboratory-confirmed cryptosporidiosis reported to the Office of Epidemiology between July 1 and October 1, 1986. If more than one person with laboratory-confirmed cryptosporidiosis was identified within a household or day care group, only the one with the earliest onset of symptoms was included in the study.

Controls were matched for age, sex, and neighborhood using a reverse telephone directory. The home of the case-patient was identified in the directory. The homes listed on either side of the case-patient were contacted by telephone alternately until suitable controls were identified. Case-patients 10 years old or younger were matched within two years of age, and those over 10 years were matched within five years of age. A control could not have had diarrhea in the four weeks before the onset of disease in the case-patient to which he/she was matched. Twenty-two of the 24 index cases were matched with two controls each. Two index cases were matched with only one control.

A standard questionnaire, administered via telephone to both case-patients and controls, requested information on size of household, day care attendance or contact, travel, surface water exposure, pets and domestic animal exposure during the four weeks before onset of illness in the case-patient.

Two surface water samples were collected on October 27, 1986 from a lake in southern New Mexico and a lake in Arizona where three of the case-patients had been exposed to surface water. These samples were tested for *Cryptosporidium* and *Giardia* using a fluorescent labeled monoclonal antibody technique.[10,11]

Using the matched triplets or pairs, we calculated Mantel-Haentzel chi-square, odds ratios (OR), and 95% confidence intervals (CI) and fitted a conditional logistic regression to determine which variables were independently associated with infection with *Cryptosporidium*.

Results

Of the 56 laboratory-confirmed cases of cryptosporidiosis in Bernalillo County reported between July 1 and October 1, 26 met the definition of a case-patient. Two (8 percent) of the cases were lost to follow up (a 1-year-old female who attended a day care center and a 43-year-old female). Of the 30 cases excluded from the study, two were patients with acquired immune deficiency syndrome who died during the course of the study, 10 were household

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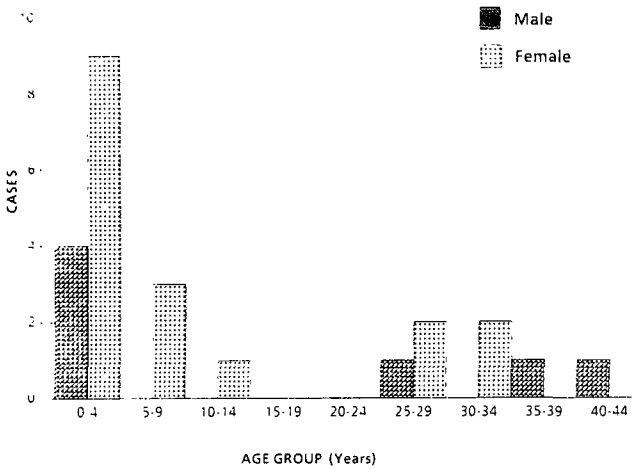

FIGURE 1—Cryptosporidiosis, by Age Group and Sex, New Mexico, 1986

contacts of case-patients, and 18 were day care contacts of case-patients.

The 24 remaining case-patients ranged in age from 4 months to 44 years with a median age of 3 years. Figure 1 shows the age and sex distribution of the index cases. Seventeen of the cases were 10 years of age or younger; 13 of whom were females; 17 of all 24 cases were female. Thirteen (54 per cent) of the case-patients were White and 11 (46 per cent) were Hispanic. Controls showed similar demographic characteristics.

Case-patients had onset of symptoms from May 28 to September 2, 1986 (Figure 2). The duration of symptoms ranged from five to 60 days with a median of 21 days. The most frequent symptom (Table 1) was diarrhea followed by flatulence and abdominal pain.

Matched set analysis identified two possible sources of infection: surface water (rivers, lakes and streams) and day care centers (Table 2). Five case-patients drank untreated surface water, two in Mexico, and three in southern Colorado and New Mexico; none of the controls drank surface water. Case-patients reported more swimming in surface water in the four weeks prior to illness. The time between exposure to surface water (swimming or drinking) and onset of illness ranged from four to 21 days with a median of seven days. Fourteen (82 per cent) of 17 household members with exposures to surface water similar to the case-patients also became ill with diarrhea. These household members became ill within two to seven days of the onset of illness in the case-patient.

TABLE 1—Frequency of Reported Symptoms of Cryptosporidiosis, New Mexico, 1986 (N = 24)

Symptom	No	%
Diarrhea	23	96
Flatulence	20	79
Abdominal Pain	17	67
Nausea	15	58
Fever	13	54
Vomiting	12	46
Abdominal distention	12	46

Case-patients were substantially more likely to attend a day care center where other children were ill with diarrhea than were their matched controls (Table 2). Attendance at day care centers where no other children were ill was not related to risk.

The logistic regression model for multivariate analysis included all variables except drinking surface water, which did not vary. Infection was associated with swimming in surface water, attending a day care center, camping, and having a pet which was less than six months of age or was ill, with odds ratios all greater than 3.0. Having a pet over six months of age was associated with a decreased risk of disease. All of these estimates were unstable, with 95% confidence limits including 1.0.

Among the 24 case-patients, eight were exposed to surface water, eight were day care center attendees or employees, six were household contacts of a day care center attendee, and eight had young and/or ill pets. Four had none of these exposures.

The surface water samples from New Mexico and Arizona where cases had been exposed did not yield *Cryptosporidium* or *Giardia* on testing.

Two of the 24 index cases were coinfected with *Giardia*.

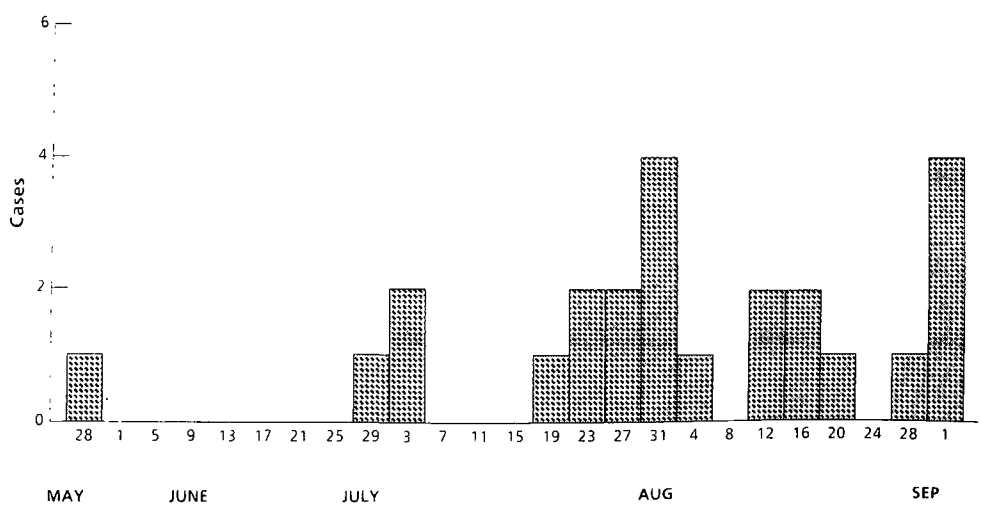

FIGURE 2—Cryptosporidiosis, by Date of Onset, New Mexico, May 28–September 4, 1986

TABLE 2—Exposures Associated with Cryptosporidiosis

Exposure	No. of Cases Exposed	No. of controls exposed 0	1	2	Odds Ratio	95% Confidence Interval
Drink surface water	0	17 (2)	0	0	00	2.44, 00
	1	5	0	0		
Swim in surface water	0	14	3	0	3.7	1.02, 13.5
	1	4 (2)	1	0		
Attend a day care center where other children were ill	0	15 (1)	1	0	13.1	2.58, 66.4
	1	6 (1)	0	0		
Household contact of day care center attendee	0	12 (1)	4	0	3.0	0.91, 9.85
	1	5 (1)	1	0		

Numbers in parentheses represent cases with only one control.

One of them attended a day care center. The other had no apparent risk factors for cryptosporidiosis or giardiasis.

Discussion

Person-to-person transmission of cryptosporidiosis is well recognized, especially in day care centers.[5,12] Lack of hygiene and frequent mouthing of objects among diapered babies and toddlers can lead to the rapid spread of this and other enteric infections in day care centers and homes.[13] This study confirmed the association between attending a day care center, especially where other children are ill, and cryptosporidiosis.

Cryptosporidiosis has also been found to be a cause of traveler's diarrhea, particularly in people traveling to less developed countries.[2,14] In this study, two (8 per cent) of the index cases had recently traveled to Mexico; both of these case-patients also drank untreated surface water in Mexico.

Although puppies and kittens have been shown to excrete *Cryptosporidium*,[4] these pets have not previously been demonstrated to transmit cryptosporidiosis to humans. Contact with a young and/or ill pet was associated with an increased risk of disease in this study, but the numbers are too small to be convincing. Nevertheless, this observation is in keeping with what is known of cryptosporidiosis in domestic animals: young animals contract cryptosporidiosis more often than older animals, and the severity and duration of illness is inversely related to the age of the animal.[1,15]

This study suggests for the first time that drinking surface water may be a mode of transmission of cryptosporidiosis in the United States. This is feasible since waterborne cryptosporidiosis has been previously demonstrated in a community in Texas due to a contaminated artesian well.[6] The plausibility of waterborne transmission is increased by the time relationship between exposure to surface water and onset of illness. The median incubation period of seven days in those case-patients exposed to surface water is consistent with what is known of the incubation period for cryptosporidiosis.[16]

Cryptosporidium sp. has been found in a broad variety of both domestic and wild animals. Cattle and sheep appear to be important hosts, but mice, snakes, rabbits, and deer have also been found to excrete the organism.[4,15] Much of the recreational water in New Mexico and other western states drains land which has multiple uses including the grazing of livestock. Livestock could contaminate surface water with *Cryptosporidium* by direct deposit of feces in the water or via surface runoff washing animal feces into the water. Similarly, contamination of the water by wild animal feces might also play an important role. New Mexico received heavier than normal rainfall during the summer months of 1986, a factor which may have promoted contamination of surface water.

The finding of an elevated risk of cryptosporidiosis among persons who had recreational contact with, but did not drink surface water, supports waterborne transmission of the disease. Recreational surface water contact, specifically swimming, has been shown to be a risk factor for other enteric infections.[17,18] Swimmers become infected by swallowing small amounts of contaminated water, especially following submersion. The infective dose of *Cryptosporidium* is unknown, but is probably small, because of the organism's ability to complete its life cycle in a single host and to reinitiate the developmental cycle in that host.[4] A small infective dose for cryptosporidiosis would facilitate transmission by water contact without frank water consumption.

Our studies of surface water in New Mexico were limited in scope, but failed to demonstrate the presence of *Cryptosporidium*. These studies were done two months after the outbreak and probably represent a failure to detect intermittent, low-level, or seasonal contamination. Additionally, the samples were taken from a large lake and it was difficult to determine the exact site of exposure. Studies of surface water conducted in Arizona showed that there was widespread contamination of streams with *Cryptosporidium* during the summer of 1986,[11] but that most of the water sources did not contain *Cryptosporidium* during the fall months.*

Cryptosporidiosis occurs more commonly in summer and fall in these and other data.[3,4] Increased outdoor activity during the summer and early fall with drinking of or swimming in surface water may partially explain the seasonal variation.

Coinfection with *Giardia lamblia* has been found in 2 per cent to 23 per cent of cryptosporidiosis cases in previously described outbreaks[19–21] and 8 per cent in this study. The coincidental occurrence of two parasitic infections suggests a common mode of transmission. Person-to-person transmission of both giardiasis and cryptosporidiosis in day care centers has been well described.[12] Waterborne transmission of giardiasis from surface water is also well known.[22,23] Our

* Unpublished data, CR Sterling, University of Arizona, 1986.

study suggests that waterborne transmission of cryptosporidiosis may explain coinfection with these two parasites, even though neither of the coinfections in this study occurred in persons who had exposure to surface water.

The median duration of diarrhea in the study population was longer than the three to 14 days described by others,[1,17,25] perhaps because patients who had stool samples submitted for ova and parasite examination had diarrhea that lasted longer than three to five days and did not respond to usual symptomatic treatment.

The predominance of young children found here has been seen in other studies, and follows the same pattern seen in domestic animals. In addition, young children are more likely to come to medical attention earlier in the course of a diarrheal illness than adults and more likely to be tested for the cause of their diarrhea. The explanation for the predominance of females seen in this group of children is not clear and may reflect only incomplete case ascertainment.

Further study of the role of surface water in transmission of cryptosporidiosis is clearly needed. Meanwhile, boiling of surface water rather than chemical disinfection may be prudent, since *Cryptosporidium* is resistant to most commonly used chemical disinfectants including chlorine and iodine,[15,24] but oocysts are destroyed by heating above 65°C for 30 minutes.[24] Boiling also destroys other organisms, including *Giardia*, that may be in the water. We also recommend that physicians consider cryptosporidiosis in the differential diagnosis of persons with diarrhea and a history of surface water contact.

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REFERENCES

1. Navin TR, Juranek DD: Cryptosporidiosis: Clinical, epidemiologic, and parasitologic review. Rev Infect Dis 1984; 6:313–327.
2. Navin TR: Cryptosporidiosis in humans: Review of recent epidemiologic studies. Eur J Epidemiol 1985; 1:77–83.
3. Mata L: *Cryptosporidium* and other protozoa in diarrheal disease in less developed countries. Pediatr Infect Dis 1986; 5:S117–S130.
4. Current WL: Cryptosporidiosis. J Am Vet Med Assoc 1985; 187:1334–1338.
5. Centers for Disease Control: Cryptosporidiosis among children attending daycare centers—Georgia, Pennsylvania, Michigan, California, New Mexico. MMWR 1984; 33:559–601.
6. D'Antonio RG, Winn RE, Taylor JP, *et al:* A waterborne outbreak of cryptosporidiosis in normal hosts. Ann Intern Med 1985;103:886–888.
7. Hunt DA, Shannon R, Palmer SR, Jephcott AE: Cryptosporidiosis in an urban community. Br Med J 1984; 289:814–816.
8. Wolfson JS, Richter JM, Waldron MA, Weber DJ, McCarthy DM, Hopkins CC: Cryptosporidiosis in immunocompetent patients. N Engl J Med 1985; 312:1278–1282.
9. Ma P, Soave R: Three-step stool examination for cryptosporidiosis in 10 homosexual men with protracted watery diarrhea. J Infect Dis 1983; 147: 824–828.
10. Garcia LS, Brewer TC, Bruckner DA: Fluorescent detection of *Cryptosporidium* oocysts in human fecal specimens using monoclonal antibodies. J Clin Microbiol 1987; 25:119–121.
11. Madore MS, Rose JB, Gerba CP, Arrowood MJ, Sterling CR: Occurrence of *Cryptosporidium* oocysts in sewage effluents and selected surface waters. J Parasit 1987; 73:702–705.
12. Pickering LK, Bartlett AV, Woodward WE: Acute infectious diarrhea among children in day care: Epidemiology and control. Rev Infect Dis 1986; 8:539–547.
13. Pickering LK, Evans DG, DuPont HL, Vollet JJ, Evans DJ: Diarrhea caused by *Shigella*, rotavirus and *Giardia* in day-care centers: Prospective study. J Pediatr 1981; 99:51–56.
14. Sterling CR, Seegar K, Sinclair NA: *Cryptosporidium* as a causative agent of traveler's diarrhea. J Infect Dis 1986; 153:380–381.
15. Tzipori S: Cryptosporidiosis in animals and humans. Microbiol Rev 1983; 47:84–96.
16. Jokipii L, Jokipii AMM: Timing of symptoms and oocyst excretion in human cryptosporidiosis. N Engl J Med 1986; 315:1643–1647.
17. Centers for Disease Control: CDC *Shigella* surveillance. Atlanta: CDC, 1976; 37:4.
18. Makintubee S, Mallonee J, Istre G: Shigellosis outbreak associated with swimming. Am J Public Health 1987; 77:166–168.
19. Skeels MR, Sokolow R, Hubbard CV, Foster LR: Screening for coinfection with *Cryptosporidium* and *Giardia* in Oregon public health clinic patients. Am J Public Health 1986; 76:270–273.
20. Wolfson JS, Hopkins CC, Weber DJ, *et al:* An association between *Cryptosporidium* and *Giardia* in stool. N Engl J Med 1984; 310:788.
21. Jokipii L, Pohjola S, Jokipii AMM: *Cryptosporidium*: A frequent finding in patients with gastrointestinal symptoms. Lancet 1983; 2:358–361.
22. Dykes AC, Juranek DD, Lorenz RA, *et al:* Municipal waterborne giardiasis: An epidemiologic investigation. Beavers implicated as a possible reservoir. Ann Intern Med 1980; 92:165–170.
23. Lopez CE, Dykes AC, Juranek DD, *et al:* Waterborne giardiasis: A communitywide outbreak of disease and a high rate of asymptomatic infection. Am J Epidemiol 1980; 112:495–507.
24. Soave R, Armstrong D: *Cryptosporidium* and cryptosporidiosis. Rev Infect Dis 1986; 8:1012–1023.
25. Current WL, Reese NC, Ernst JV, Bailey WS, Heyman MB, Weinstein WM: Human cryptosporidiosis in immunocompetent and immunodeficient persons: Studies of an outbreak and experimental transmission. N Engl J Med 1983; 308:1252–1257.

III. Investigation of Waterborne Disease Outbreaks

AMERICAN JOURNAL OF Public Health

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Community Health Effects of a Municipal Water Supply Hyperfluoridation Accident

LYLE R. PETERSEN, MD, DIANE DENIS, MPH, DAVID BROWN, DSC, JAMES L. HADLER, MD, MPH, AND STEVEN D. HELGERSON, MD, MPH

Abstract: For 12 hours, excess hydrofluorosilicic acid was diverted to a 127-home community water supply. Fluoride levels peaked at 51 parts per million (ppm). Water acidification caused copper to leach from the domestic plumbing; raising copper levels to 25–41 ppm. Fifty-two (33 per cent) of those who drank hyperfluoridated water developed mild gastroenteritis. Vomiting was uncommon and symptom onsets usually occurred >30 minutes after drinking water; suggesting that fluoride, rather than copper, caused illness. Skin contact with hyperfluoridated water caused itching and skin rashes. (*Am J Public Health* 1988; 78:711–713.)

Introduction

Hyperfluoridation accidents in public water supplies are uncommon[1–5] and accidents in municipal water supplies have been reported only twice previously.[4,5] This report describes the public health effects of a third municipal water supply hyperfluoridation accident that occurred in a residential Connecticut community.

Background

The community has 127 homes. Its water source is a nearby treatment plant which also supplies water to three distant larger metropolitan areas. The community is on a "dead-end" portion of the water distribution system. The street water mains are cement lined while feeder lines from the street to each house are copper as is the piping within the houses.

At the treatment plant, hydrofluorosilicic acid (H_2SiF_6) is injected into the water supply. At approximately 3:00 pm on March 11, 1986, an inadvertently opened valve began to divert hydrofluorosilicic acid that normally would have been injected into water supplying the community and distant metropolitan areas, solely into the community's water supply. Hyperfluoridated water would have reached the domestic taps at approximately 6:00 pm (beginning of exposure period, time = 0 hours). At +1 hours (7:00 pm) residents began notifying water company personnel that the water tasted abnormal and turned blue on contact with soap, and of itching and gastrointestinal symptoms. At +1 and +4 hours (7:00 and 10:00 pm), household tap water samples revealed fluoride and copper concentrations >40 times normal (fluoride 42–51 ppm [normal 1.0 ppm], copper 25–41 ppm [normal 0.03 ppm]).

At +10 hours (4:00 am), a sample of water from a water main had fluoride and copper concentrations of 50 ppm and 0.03 ppm, respectively. The water mains were then flushed. Beginning at +12 hours (6:00 am), residents were told not to drink or bathe in the water and to discard ice or beverages made with tap water.

From the Division of Field Services, Epidemiology Program Office, Centers for Disease Control (Dr. Petersen); the Preventable Diseases Division, State of Connecticut Department of Health Services (Ms. Denis, Dr. Brown, Dr. Hadler); Office of the Director, Centers for Disease Control (Dr. Helgerson); and the Yale University School of Medicine, Department of Epidemiology and Public Health (Dr. Helgerson).

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Methods

Epidemiologic Investigation

On March 15, 1986, a door-to-door survey of the 127 community households was conducted. Data were obtained from at least one adult (>16 years in age) to determine each household member's water consumption, dermal exposure to water, and symptoms.

The exposure period was considered to have been +0 hours to +12 hours. The quantity of ingested hyperfluoridated water was estimated as the number of glasses of tap water consumed during the exposure period plus the number of glasses of beverages made from tap water during that period. The latent interval was defined as the time from consumption of the last glass of hyperfluoridated water to the illness onset. A person was considered dermally exposed to the hyperfluoridated water if that person bathed or showered at home during the exposure period.

The outbreak period was defined from 0 to +54 hours. A case of gastrointestinal irritation was a resident with onset during this period of any of the following symptoms: diarrhea (≥2 watery stools in a 24-hr period), abdominal cramping, severe nausea, or vomiting. A case of skin irritation was a resident with onset of unusual itching during the outbreak period.

Statistical relations between water consumption or dermal exposure and illness were determined by the method of related ratios to control for household.[6,7] The dose-response relation of water consumption and gastroenteritis was assessed by the Mantel Chi square test for trend[8]; other differences were assessed by the Chi square test. The effective dose of hyperfluoridated water that would be expected to cause gastroenteritis in 50 per cent of a population (ED_{50}) was calculated by the log probit method.[9]

Results

Information concerning 321 persons was gathered from 86 (68 per cent) of the 127 households. Representatives from two households refused interviews and in 39 households no adult was available for interview on the survey day. Abnormal taste or color of water was reported in 62 per cent (53/86) of households.

Gastrointestinal Illness

Gastrointestinal symptom histories were obtained for 312 persons, 55 (18 per cent) of whom were cases; symptoms included: abdominal cramping (66 per cent), nausea (62 per cent), headache (49 per cent), diarrhea (42 per cent), vomiting (13 per cent), diaphoresis (12 per cent), and fever (4 per cent). The onset of illness for the 46 persons with known time of symptom onset is depicted in Figure 1. The median duration of gastroenteritis symptoms was 5.5 hours with a range of 1 to 60 hours. No person sought medical evaluation for gastroenteritis symptoms.

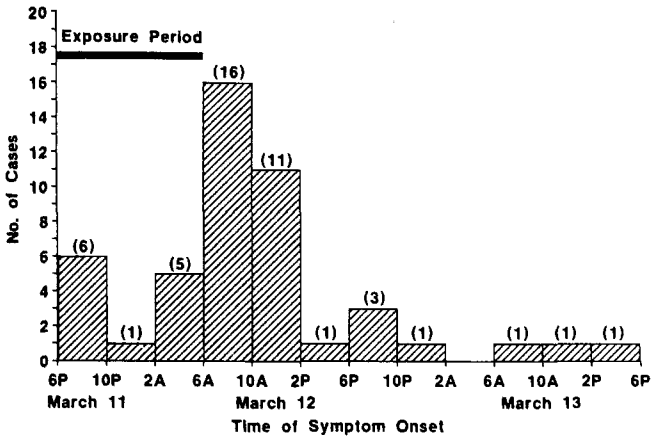

FIGURE 1—Gastroenteritis, by Time of Symptom Onset, Connecticut Community, March 11–13, 1986

Water consumption and symptom histories were available for 301 persons. Of the 160 persons who drank water, 52 (33 per cent) had gastroenteritis compared to only two (1.4 per cent) of the 141 persons who did not drink water (relative risk = 23; 95 per cent confidence intervals = 5.7, 92.4). Information about latent interval was available for 37 persons and, for those persons, the median latent interval was two hours (Figure 2). Only four persons (11 per cent) had symptom onsets <30 minutes after drinking water. Attack rates were similar by gender, age, and location of residence.

Attack rates by quantity of tap water consumed for 160 persons for whom data were available were: 29 per cent (33/114), 1–2 glasses; 31 per cent (11/36), 3–4 glasses; and 80 per cent (8/10), ≥5 glasses. The calculated ED_{50} was 2.7 glasses of water (95 per cent CI = 1.8, 4.1). This corresponded to 33.8 mg of fluoride and 20.2 mg of copper if an average glass of water was 250 cc and contained 50 ppm of fluoride and 30 ppm of copper.

Skin Irritation

Of the 300 persons whose skin irritation histories were obtained, 30 (10 per cent) reported unusual itching, with a duration of 2 to 62 hours. Of these 30 persons, 12 reported skin rash compared to only two of 270 persons who did not report itching. No person had a skin rash on the survey day.

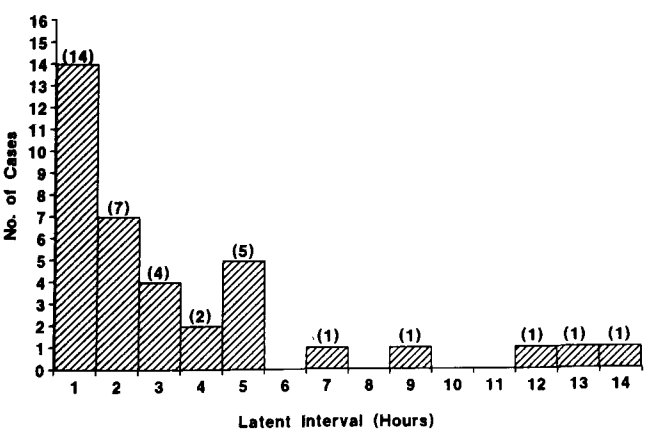

FIGURE 2—Interval from Water Consumption to Onset of Gastroenteritis, Connecticut Community, March 11–13, 1986

TABLE 1—Cases of Skin Irritation by Bathing or Showering Status, Connecticut Community, March 11–12, 1986

Water Exposure	Cases	Total*	Attack Rate %	Rate Ratio	95% Confidence Intervals
Bath	5	19	26	4.2	1.7–10.7
Shower	13	88	15	2.4	1.1–5.0
Neither	11	176	6	1.0	
Unknown	1	17			
Totals	30	300			

*21 persons had unknown skin irritation histories.

Persons dermally exposed to hyperfluoridated water in a shower or bath (Table 1) were 2.7 times as likely to have reported itching than unexposed persons (95 per cent CI = 4, 5.3). Persons who reported itching, but who had not bathed or showered, had other water exposures such as dishwashing, and their itching was localized to the area of water contact.

Discussion

This hyperfluoridation accident was caused by diversion of excess hydrofluorosilicic acid into a community water supply. The resultant water acidification substantially elevated water copper levels by solubilizing copper compounds coating the domestic copper service lines, as demonstrated by elevated copper levels in tap water and normal levels in water main water. In the previous municipal water supply outbreak caused by excess hydrofluorosilicic acid, high iron levels resulted from contact with iron pipes.[4]

Although one-third of those who drank the water became ill, symptoms were mild and of short duration. The ED_{50} of 2.7 glasses of water in this outbreak corresponded to symptom-producing doses of copper and fluoride observed in other studies. Copper ingestion causes vomiting usually within 10 minutes,[10] and outbreaks have occurred in water supplies containing from 4–430 ppm of copper[10–13] and with as little as a 5 mg ingested dose.[10]

Low-dose fluoride ingestion causes nausea, vomiting, abdominal cramping, and diarrhea,[1,2,4,14] and outbreaks have occurred in water supplies with levels from 30 to >1,000 ppm.[1–5] Symptoms occur with a 5 mg ingested dose.[15] Both copper and fluoride may have had additive effects in this outbreak; however, the infrequency of vomiting and symptoms occurrences <30 minutes after water ingestion suggested fluoride toxicity.

Skin irritation symptoms with a hyperfluoridation accident have not been reported and, in this outbreak, could have been caused by fluoride, copper, or the water's acidity. Although hydrofluorosilicic acid produces burns in high concentrations,[16] its cutaneous effects in low concentrations are unknown. Dermally applied copper salts can produce itching and skin rash[17] but the cutaneous effects of copper at levels recorded in this outbreak are unclear.

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REFERENCES

1. Centers for Disease Control: Acute fluoride poisoning—North Carolina. MMWR 1974; 23:199.
2. Hoffman R, Mann J, Calderone J, Trumbull J, Burkhart M: Acute fluoride poisoning in a New Mexico elementary school. Pediatrics 1980; 65:897–900.
3. Craun GF: Waterborne outbreaks in the United States, 1971–78. Am Water Works Assoc 1980 Annual Conference Proceedings. Denver: Am Water Works Assoc, 1980.
4. Leland DE, Powell KE, Anderson RS: A fluoride overfeed incident at Harbor Springs, Michigan. J Am Water Works Assoc 1980; 72:238–243.
5. Centers for Disease Control: Fluoride intoxication in a dialysis unit—Maryland. MMWR 1980; 29:134–136.
6. Cochran WG: Sampling Techniques. New York: John Wiley, 1977.
7. Mendenhall W, Ott L, Schaeffer RL: Elementary Survey Sampling. North Scituate, MA: Duxbury Press, 1979.
8. Mantel N: Chi-square tests with one degree of freedom: Extensions of the Mantel-Haenzel procedure. J Am Stat Assoc 1963; 58:690–700.
9. Gad SC, Weil CS: Statistics for toxicologists. *In:* Hayes AW (ed): Principles and Methods of Toxicology, Vol 2. New York: Raven Press, 1984; 273–320.
10. Gosselin RE, Hodge HC, Smith RP, Gleason MN (eds): Clinical Toxicology of Commercial Products, 5th Ed. Baltimore: Williams and Williams, 1984.
11. Wyllie J: Copper poisoning at a cocktail party. Am J Public Health 1957; 47:617.
12. Nicholas PO: Food-poisoning due to copper in the morning tea. Lancet 1968; 2:40–42.
13. Hopper SH, Adams, HS: Copper poisoning from vending machines. Public Health Rep 1958; 1:910–914.
14. Semple AB, Parry WH, Phillips DE: Acute copper poisoning: an outbreak traced to contaminated water from a corroded geyser. Lancet 1960; 2:700–701.
15. Spoerke DG, Bennett DL, Gullekson D: Toxicity related to acute low dose sodium fluoride ingestions. J Fam Pract 1980; 10:139–140.
16. Burke WJ, Hoegg VR, Phillips RE: Systemic fluoride poisoning resulting from a fluoride skin burn. JOM 1976; 15:39–41.
17. Clayton GD, Clayton FE (eds): Patty's Industrial Hygiene and Toxicology, 2nd Ed., Vol II. New York: Wiley and Sons, 1963.

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AN EPIDEMIC OF GASTROENTERITIS TRACED TO A CONTAMINATED PUBLIC WATER SUPPLY

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Weissman, J. B., G. F. Craun, D. N. Lawrence, R. A. Pollard, M. S. Saslaw and E. J. Gangarosa (CDC, Atlanta, GA 30333). An epidemic of gastroenteritis traced to a contaminated public water supply. *Am J Epidemiol* 103: 391–398, 1976.

Between January 1 and March 15, 1974, approximately 1200 cases of acute gastrointestinal illness occurred in Richmond Heights, Florida, a residential community of 6500 in south Dade County. Over one-third of all families in the area had at least one member affected. The findings of 10 culture-proven cases of shigellosis among those who became ill and clinical signs and symptoms in the others suggest that most of the other cases that were not cultured may have been shigellosis also. Epidemiologic investigation showed that consumption of tap water was associated with illness in the initial cases of affected families. Evaluation of the Richmond Heights public water supply disclosed numerous inadequacies in both design and operation. One of the wells providing water to the community was continuously contaminated with excessive levels of fecal coliforms from a nearby septic tank, and a breakdown in chlorination on January 14–15 caused approximately 1 million gallons of inadequately chlorinated water from the contaminated well to be distributed to the community 48 hours before the epidemic began. Correction of deficiencies in the water plant was undertaken by the utility company; the residents of Richmond Heights were instructed to boil their drinking water or to use commercially bottled water pending completion of corrective measures. A full scale study is planned for all similar public water supplies in Dade County.

disease outbreaks; gastroenteritis; Shigella infections; water pollution

INTRODUCTION

In March 1974, routine investigation of 10 cases of shigellosis in Richmond Heights, Florida, a residential suburb of Miami in south Dade County, indicated that up to 1000 clinical cases of an illness characterized by diarrhea and abdominal pain had occurred in residents between January 1 and March 15, 1974. Subsequent studies developed evidence that illness resulted from massive contamination of the area's public water supply. This report describes the results on these investigations.

EPIDEMIOLOGIC METHODS

To evaluate the true extent of disease in Richmond Heights, we reviewed county records from 1970–1974 for cases of shigellosis and subsequently undertook a survey of 75 households in the area (approxi-

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mately 5 per cent of all households) chosen by a two-stage cluster sampling technique (1).

A survey form was developed for use by public health nurses in the field. The name, age, sex, and school or occupation of everyone in the household was listed. The respondent was asked about illness, defined as diarrhea and abdominal pain since January 1, 1974, in each person. For everyone who had been ill, the date of onset, duration of symptoms, symptom complex, and details of medical treatment were obtained. Each family member, ill or well, was asked to estimate his daily water consumption, indicating whether he drank tap or bottled water.

Each respondent furnished additional information: whether a private well was present on the property, and if so, if it was used only for irrigation or as a source of potable water, and whether there had been recent septic tank malfunction. A variety of questions were asked to uncover possible common exposures, such as the source of the household's meats and vegetables, restuarants frequented, church and community activities, and contacts with people who had similar illnesses. Respondents with ill family members were also asked how they thought illness was introduced into the home.

Since the endemic level of diarrheal diseases in Richmond Heights was unknown and could not be determined reliably due to time lapse and unusual intervening events, we selected a nearby community to serve as a control. After reviewing a variety of socioeconomic characteristics of approximately 2 dozen communities in Dade County, we chose Bunche Park about 15 miles (24 km) north of Richmond Heights, because its social, racial, and economic profile most closely approximated that of Richmond Heights. A survey similar to the one performed in Richmond Heights was conducted in Bunche Park.

Florida Water and Utilities, Inc., the company operating the Richmond Utilities Company, provided records of water usage, termination of services to consumers because of non-payment of bills, blueprints of the water distribution system, and daily operational records. The Dade County Department of Public Health provided records of coliform counts on routine water samples obtained by health department sanitarians.

Laboratory methods

Rectal swabs and stool specimens were processed by the Epidemiologic Services Laboratory Branch, Center for Disease Control (CDC). *Shigella sonnei* isolates from Dade County were forwarded to CDC for biochemical confirmation, antibiotic sensitivity testing, and colicin typing. Water samples collected during the investigation were analyzed by the State's Miami Regional Laboratory, at CDC and at the Water Supply Research Laboratory, Environmental Protection Agency (EPA). Water samples were analyzed for total and fecal coliforms by the membrane filter technique; standard plate counts were performed on several samples to provide an estimate of total bacterial population. Fluorescein was used in dye studies, and fluorescence was measured spectrophotometrically.

Results

Epidemiologic investigations in Dade County

Review of county records of culture-proven cases of *Shigella sonnei* infection for the five-year period 1970–1974 disclosed that a statistically significant excess number of isolates were reported in the first 10 weeks of 1974 as compared with the four preceding years ($p < .05$) (table 1). Without the 10 cases reported from Richmond Heights, however, the total in the first 10 weeks of 1974 was within 2 SD of the mean

TABLE 1
Cases of shigellosis, Dade County, Florida 1970-1974

Year	Annual total	First 10 weeks
1970	108	17
1971	76	12
1972	103	15
1973	75	10
1974	148	29
Mean, 1970-1973	90.5	13.5

for the same time period during the four preceding years. Of the 29 reported cases, those in Richmond Heights occurred significantly earlier than cases elsewhere in Dade County. Seven of 10 cases in Richmond Heights occurred in the first five weeks of the 10-week period, compared with none of eight cases in communities adjacent to Richmond Heights ($p = .004$) or with three of 16 cases elsewhere in Dade County ($p = .006$).

Shigella sonnei isolates from Richmond Heights differed in their antibiotic resistance and colicin-typing patterns from all other isolates in Dade County during the period January–March 1974. All those from Richmond Heights patients had an untypable colicin pattern and were sensitive to all antibiotics tested. Four patients from elsewhere in Dade County had untypable isolates that were antibiotic resistant. Four additional colicin patterns (none were the pattern of an established colicin "type") were observed among six of the Dade County isolates, but none was an isolate from a patient in Richmond Heights. There were no shigella isolates from Bunche Park during the time period studied.

Interviewers succeeded in contacting 73 of 75 households in Richmond Heights and 70 of 75 in Bunche Park. In Richmond Heights, data were obtained for 353 persons, and in Bunche Park for 292. Twenty-seven of 72 households (37.5 per cent) in Richmond Heights had members who were ill, compared with 11 of 70 (15.7 per cent) in Bunche Park ($\chi^2 = 7.52$, $p < .01$).

Sixty-seven of 353 people (19.0 per cent) in Richmond Heights were ill, compared with 16 of 292 (5.5 per cent) in Bunche Park ($p < .01$, t test). Thus, the incidence of diarrheal disease in Richmond Heights was significantly greater than in a control community during the time period examined. Extrapolation of these data to the community as a whole would indicate that 1256 ($\pm$450) cases of diarrheal illness occurred in Richmond Heights in the first 10 weeks of 1974 (95 per cent confidence interval).

Analysis of the distribution of cases in the Richmond Heights survey population disclosed a mean age of patients of 17.2 years, close to the mean of 20.9 years of household members who were not ill; attack rates were distributed with relative uniformity throughout all age groups (table 2). The age distribution of cases was also slightly different from that of shigellosis in the United States (2).

Cases of diarrheal disease in the survey population began to occur in epidemic numbers on January 17, 1974, peaking about one month later and rapidly falling off afterward (figure 1). The time course of cases in the survey population paralleled that of the culture-proven cases of *S. sonnei* in Richmond Heights. Illness in both survey populations was characterized by fever, nausea, vomiting, and diarrhea; overall illness in Richmond Heights was characterized by a greater number of specific symptoms than in either Bunche Park or among the culture-proven cases (table 3). Symptoms of patients in the Richmond Heights survey population differed significantly from those in Bunche Park patients in that there was a significantly higher incidence of tenesmus, fever, and vomiting, but did not differ significantly from the culture-proven cases. In none of the symptoms noted was the incidence higher for patients in the Bunche Park survey

TABLE 2

Age-specific illness in Dade County survey populations and in households of patients with culture-positive shigellosis, 1974

Age group	Culture-proven shigellosis			Survey populations					
				Richmond Heights			Bunche Park		
	Ill (No.)	Not ill (No.)	Attack rate (%)	Ill (No.)	Not ill (No.)	Attack rate (%)	Ill (No.)	Not ill (No.)	Attack rate (%)
0–4	0	1	.00	7	15	.32	0	31	.00
5–9	5	2	.71	16	31	.34	1	24	.04
10–19	4	11	.27	18	102	.15	4	75	.05
20–29	1	6	.14	5	32	.14	3	21	.13
30–39	2	4	.33	8	30	.21	2	15	.12
40–49	0	5	.00	2	35	.05	4	46	.08
50–59	1	0	1.0	7	18	.28	2	28	.07
60–69	1	4	.20	4	17	.19	0	23	.00
70+	2	0	1.0	0	2	.00	0	11	.00
Unknown	0	0	.00	0	4	.00	0	2	.00
Total	16	33	.33	67	286	.19	16	276	.05

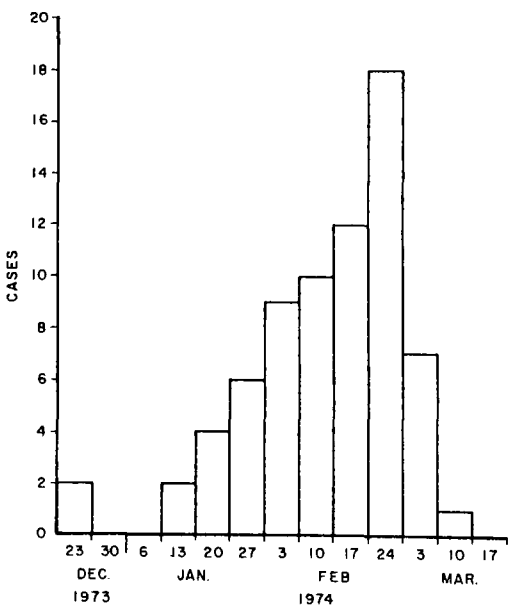

FIGURE 1. Gastroenteritis by week of onset. Richmond Heights survey population, January–March 1974.

TABLE 3

Symptom complex in survey populations, by per cent, Dade County, 1974

Symptom	Culture-proven shigellosis	Survey populations	
		Richmond Heights	Bunche Park
Fever	80.0	70.2	33.3
Tenesmus	53.3	78.6	38.5
Nausea	50.0	76.9	53.8
Vomiting	46.7	63.2	28.6
Headache	38.5	66.7	53.8
Bloody diarrhea	23.1	11.1	6.7

population than in the Richmond Heights survey group.

There were no significant correlations between illness in the Richmond Heights survey population and attendance at specific schools, churches, or clubs, particular sources of meats or vegetables, specific restaurants, or the presence of a private well; 23.9 per cent of the survey households had private wells, generally for use with sprinkler systems and not as a source of potable water. Sixty-nine per cent of households with illness knew of other households in which illness had occurred; only 9.3 per cent of households without illness knew of families with illness (χ^2 = 25.1, $p < .0001$). Many people volunteered that their drinking water had been intermittently discolored, "fishy" smelling or tasting, and had contained particulate matter. In the sample survey population as a whole, illness was not associated with water consumption. To eliminate the am-

biguity caused by households with multiple cases where some cases may have occurred by person-to-person spread as well as by a common source such as drinking water, the analysis of water-specific attack rates was recalculated, this time comparing water consumption of the initial cases only with water consumption by the non-ill members of the same households. Eighteen of 49 (36.7 per cent) who drank more than four glasses of water daily became ill; this incidence was significantly greater than 15 of 82 (19.5 per cent) who drank four glasses of water or less daily ($\chi^2 = 4.60$, $p < .05$). If seven households who drank only bottled water are also removed from analysis, the association of illness with water consumption increases further in statistical significance ($\chi^2 = 5.44$, $p < .03$). Similar analyses relating to illness in Bunche Park did not identify water as being associated with the low level of illness there.

Water system evaluation

At the time of the outbreak, Richmond Heights was served by a single public water system operated for the Richmond Utilities Company by Florida Water and Utilities, Inc. (figure 2). The average quantity of water pumped for the time in question was slightly more than 3790 kl per day. Water was pumped from several wells on the premises of the utility company and came from a relatively shallow aquifer located less than 15.2 meters below the surface. The principal well in use was a well, 6, drilled to a depth of 7.3 meters with a casing extending down to 6.1 meters. On January 31, a new and deeper well, 7, was put into service and, together with well 6, supplied the needs of the Richmond Heights residents.

Water from both wells was mixed, chlorinated and distributed. A 379 kl elevated storage tank was connected to the system in such a manner that during periods of high usage, water was pumped from the wells, and after passing the point of chlorine addition, was distributed directly throughout the community. As the demand in the distribution system slackened, the water surging from the wells under the

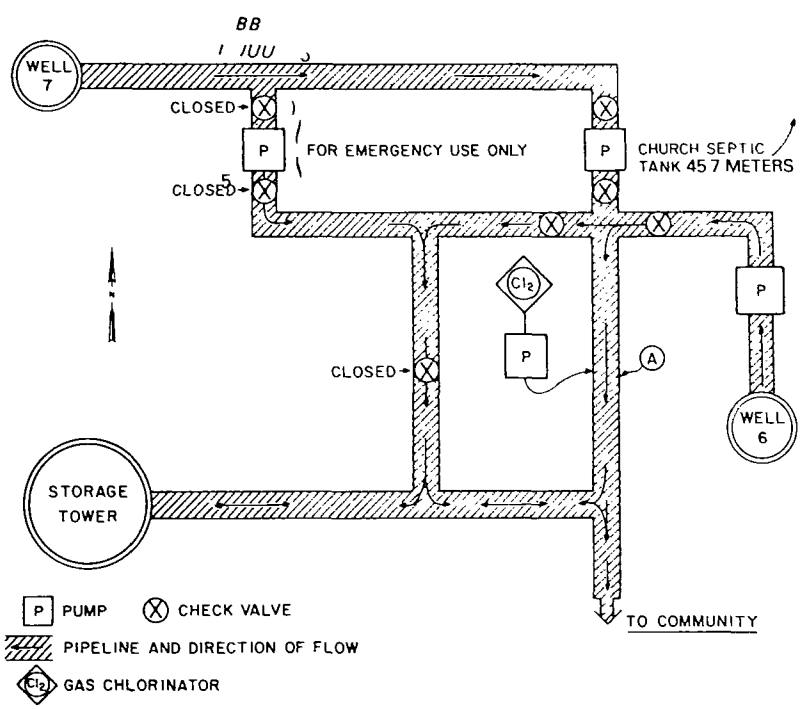

FIGURE 2. Richmond Heights water treatment plant.

influence of the pumps was intermittently diverted into the storage tank. Then, during periods of low usage, the demand was satisfied entirely by water in storage. Only when the water level in the elevated tank dropped to a predetermined level did the pumps automatically reactivate.

During the investigation, bacteriologic testing of untreated water from wells 6 and 7 revealed consistently elevated total coliform counts (>50 per ml) in samples from well 6. Fluorescein dye introduced into the flush toilet at a neighboring nursery school, which had a septic tank located approximately 38 meters from well 6, was identified in water from the well after only nine hours. Due to the porosity of the soil, the cone of influence of the pumps had been estimated to have a radius of perhaps up to 400 meters.

The method of chlorination in use consisted of the addition of chlorine from a 68 kg cylinder through a booster pump. The average amount of chlorine used per day during January, 1974, was 11.3 kg. This was successful in achieving recorded chlorine levels at the pumping station of between 1.3 ppm and 3 ppm. Similar readings were recorded for preceding and subsequent months. However, review of pumping station records revealed that on January 15, the plant operator arrived to find the chlorinator booster pump malfunctioning and only 2.3 kg of chlorine used during the preceding 24 hours. The chlorine residual recorded at the plant effluent on that day was 0. Thus, it was estimated that approximately 3790 kl of unchlorinated or, at best, inadequately chlorinated water had been distributed to the community 48 hours before the epidemic began.

Control measures

With bacteriologic evidence of continuing fecal contamination of the Richmond Heights raw water supply and epidemiologic evidence linking gastroenteritis in the community with consumption of water, the Dade County Department of Public Health issued a "boil-water emergency" alert on March 29, 1974. Announcements in newspapers and on radio and television instructed residents to boil drinking water. Public water fountains in schools and churches in the areas were closed. Personnel at the health department offices were made available to answer public inquiries. The health department also made recommendations to assure the safety of the water supply after several water system deficiencies were designated. These included the recommendations that coliform counts in raw waters to be treated exclusively by disinfection should not exceed 20 fecal or 100 total coliforms per 100 ml as measured by monthly arithmetic means (in accordance with EPA guidelines), that provisions be made to insure continuous chlorination of the raw water source, and that a chlorine contact time of at least 30 minutes be assured.

Discussion

Our investigation indicated that over 1200 individuals in Richmond Heights, about 20 per cent of the area's population, became ill in January and February 1974. A substantial body of evidence supports the association of water consumption from the public water supply with diarrheal disease, and we have concluded that the infectious agent responsible for this epidemic was waterborne.

No samples of raw water from well 6, the well supplying most of the community's water in January, were obtained during that time. However, multiple samples obtained on several days during our investigation in March were invariably contaminated with excessive numbers of fecal coliforms.

Moreover, the recovery of fluorescein dye from well 6 water shortly after introduction of the dye into a nearby sewerage system suggests that well 6 was continuously contaminated by sewage. Conse-

quently, any interruption in disinfection would inevitably have resulted in the distribution of contaminated water to the community. On January 14, two days before the epidemic began, a malfunction in the chlorinator booster pump resulted in the distribution of inadequately treated water for an entire day. Numerous potential troublespots, none of which was specifically implicated in this outbreak, included pumping raw water when chlorine cylinders were being changed, inadequate chlorine contact times, substantial pressure drops in certain areas of the distribution system, and evidence of substantial water loss through leaks or illegal connections; these factors afforded the possibility of contamination of the system in its distribution as well as its source.

Ten cases of *Shigella sonnei* infection provided the initial stimulus for this investigation. Culturing the survey population would have been both impractical and unrewarding, given the elapsed time between their illness and our investigation; symptomatology was used instead as the primary means of differential diagnosis. We cannot prove, therefore, that the clinical cases were shigellosis; however, the concordance of symptoms in the Richmond Heights survey population with the symptoms in the culture-proven cases (rather than with non-shigellosis cases in Bunche Park) and the high incidence of tenesmus, thought to be a relatively specific indicator of shigella infection, suggest that most community illness was shigellosis rather than another acute intestinal illness. Numerous reports in the literature of sewage poisoning from contaminated water have depicted a syndrome characterized by shorter duration and milder symptoms than we observed here (3-5). The possibility that some of the diarrheal illnesses may have been caused by etiologic agents other than shigella cannot be ruled out.

One year prior to this investigation CDC, EPA, and Dade County Health Department workers investigated another epidemic of waterborne disease in Dade County. In February and March 1973, 210 confirmed cases of typhoid fever occurred among 1400 residents at the South Dade Migrant Farm Labor Camp, in Homestead, Florida (6). Epidemiologic investigation at that time disclosed a highly significant correlation between water consumption and illness. Bacteriologic examination of the camp's well water supply showed the well to have been intermittently contaminated. The water was chlorinated before distribution; however, an engineering evaluation of the system's operation revealed that the single chlorine cylinder had run out of chlorine and untreated water was distributed prior to the outbreak.

Deficiencies in the water system that led to the Dade County waterborne typhoid epidemic of 1973 were duplicated in the Dade County waterborne gastroenteritis epidemic of 1974: contamination of a raw water source without a reliable system for preventing its distribution in the absence of adequate disinfection, and an unsatisfactory surveillance system to pinpoint long-standing potential hazards before their translation into real disease.

The morbidity which may occur from the waterborne transmission of enteric infection is difficult to overestimate. Less than 10 years ago, contamination of a public water system in California caused over 16,000 cases of salmonellosis in a three-week period (7). This and other outbreaks (4-6, 8, 9) underscore that contaminated public water is by no means a problem exclusively of underdeveloped or economically disadvantaged societies but rather a significant problem of contemporary urban life.

REFERENCES

1. Serfling R, Sherman I: Attribute Sampling Methods for Local Health Departments. PHS Publication No 1230, 1965, pp 46-53
2. Center for Disease Control: Shigella Surveillance

Report No 36, First and Second Quarters 1974. issued February 1975
3. Lobel HO, Bisno AL, Goldfield M, et al: A waterborne epidemic of gastroenteritis with secondary person-to-person spread. Am J Epidemiol 89:384-392, 1969
4. Werner SB, Jones PH, McCormack WM, et al: Gastroenteritis following ingestion of sewage-polluted water: An outbreak at a logging camp on the Olympic Peninsula. Am J Epidemiol 89:277-285, 1969
5. Gastroenteritis—Illinois. Center for Disease Control: Morbidity and Mortality Weekly Report 21(23):198-199, June 10, 1972
6. Feldman RE, Baine WB, Nitzkin JL, et al: Epidemiology of *Salmonella typhi* infection in a migrant labor camp in Dade County, Florida. J Infect Dis 130:334-342, 1974
7. A waterborne epidemic of salmonellosis in Riverside, California, 1965. A collaborative report. Am J Epidemiol 93:33-48, 1971
8. Gorman AE, Wolman A: Waterborne outbreaks in the United States and Canada and their significance. J Am Water Works Assn 31:225-373, 1969
9. Weibel SR, Dixon FR, Weidner RB, et al: Waterborne disease outbreaks 1946-1960. J Am Water Works Assn 56:947-958, 1964

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Chronic Diarrhea Associated with Drinking Untreated Water

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Purpose: To determine the cause of an outbreak of chronic diarrhea and to define the clinical profile of the illness.

Design: A case series of patients with chronic diarrhea and case-control and cohort studies to determine the vehicle and cause of the illness.

Setting: Rural Henderson County, Illinois.

Patients: Seventy-two patients who had onset of chronic diarrheal illness between May and August 1987. Controls were local residents and eating companions who did not have diarrheal illness. A cohort study included 80 truck drivers from a local firm.

Methods and Measurements: Nonbloody diarrhea was characterized by extreme frequency (median, 12 stools/d), marked urgency, fecal incontinence, and weight loss (mean, 4.5 kg). The median incubation period was 10 days. Nine patients were hospitalized; none died. Diarrhea persisted in 87% of patients after 6 months. Antimicrobial therapy produced no clinical improvement. No bacterial, mycobacterial, viral, or parasitic agents known to be enteropathogenic were detected in stools or implicated water. Three of five small-bowel biopsies showed mild inflammatory changes. Mild inflammation was also seen in two of nine colonic biopsies. Case-control studies implicated a local restaurant ($P = 0.0001$, odds ratio = 19) and subsequently the untreated well water served in the restaurant ($P = 0.04$, odds ratio = 9.3) as the vehicle of transmission.

Conclusions: This is the first outbreak of chronic diarrhea linked to drinking untreated water. The causative agent and pathophysiologic mechanism of the illness remain elusive.

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In 1984 and 1985, outbreaks of a distinctive chronic diarrheal illness occurred in Brainerd, Minnesota and San Antonio, Texas (1, 2). The illnesses were characterized by dramatic, urgent, watery diarrhea persisting for many months in the absence of systemic symptoms. Although epidemiologic evidence strongly implicated unpasteurized milk as the vehicle in the Brainerd outbreak, the San Antonio case-patients were not exposed to unpasteurized milk products. Extensive clinical and microbiologic studies revealed no likely pathogens in either episode, and the causative agent for "Brainerd diarrhea" remains unknown.

Physicians throughout the United States have reported sporadic cases of chronic diarrheal illness clinically similar to those seen in the Minnesota and Texas outbreaks (3), and other smaller clusters have been recognized (1). Medical evaluation of these patients is often frustrating. Although some chronic diarrhea cases have been linked with two newly defined clinicopathologic entities, collagenous colitis (4-7) and microscopic colitis (8, 9), the vehicles of transmission and causative agents are obscure.

Henderson County, Illinois is a corn and soybean farming community of 9000 people located on the eastern bank of the Mississippi River. In July 1987, a physician reported 12 cases of a distinct chronic diarrheal illness to the Henderson County Health Department. By the middle of August, the health department had accumulated information on approximately 50 patients with chronic diarrhea. Patients had been evaluated by physicians at several local hospitals; no causative agent was identified, but a local restaurant was suspected as the source. This large outbreak provided an opportunity to further delineate the clinical and pathologic profile of "Brainerd diarrhea" and to define a new vehicle of transmission, water.

Methods

A case was defined as diarrhea (three or more loose stools per day) of unknown cause lasting 4 or more weeks, with onset after 1 May 1987, in a person who had been in Henderson County after 1 April 1987. A case was considered to be restaurant-associated if the case-patient had visited the implicated restaurant during the 6 weeks before onset of illness. Cases were identified by direct patient reports to the Henderson County Health Department, by calls to local physicians and hospital infection control administrators, and by referral from known case-patients. The Illinois Department of Public Health solicited case reports from regional and county health departments. Requests were made through the newspaper and television media for persons with chronic diarrhea to notify the county health department.

Table 1. *Symptoms of Chronic Diarrheal Illness in 67 Case-Patients in Henderson County, Illinois, 1987*

Symptom	Frequency, *n(%)*
Urgency	67(100)
Borborygmi	53 (79)
Fecal incontinence	43 (64)
Flatulence	42 (63)
Cramps	30 (45)
Bloating	24 (36)
Nausea	16 (24)
Feverishness	8 (12)
Vomiting	5 (8)

Clinical Studies

Sixty-seven case-patients were interviewed regarding symptoms and treatment of their diarrheal illness. Available medical records, laboratory results, and pathology specimens from 51 case-patients were reviewed. Six months after the initial investigation a follow-up questionnaire was sent to all case-patients to assess the duration of illness.

Thirty-two case-patients submitted stool specimens; all specimens were submitted at least 4 weeks after the onset of illness. Ten specimens were cultured at the Centers for Disease Control (CDC) for *Salmonella* species, *Shigella* species, *Campylobacter jejuni/coli, Yersinia enterocolitica,* pathogenic *Vibrio* species, *Aeromonas* species, *Plesiomonas* species, *Bacillus cereus,* and *Staphylococcus aureus.* Ten colonies selected from the MacConkey agar were identified, serotyped (if identified as *E. coli*), and tested by enzyme-linked immunosorbent assay for *E. coli* enterotoxins (heat-labile and heat-stable) (10, 11). Shiga-like toxins I and II, localized and diffuse adherence, and invasiveness genes were sought with specific DNA probes (12-15). Ten bacterial colonies were also selected from blood agar plates and identified. The National Veterinary Services Laboratories cultured ten case and eight control stools for mycobacterial species, including *Mycobacterium paratuberculosis* (16). Ten stools were cultured for yeast. Ova and parasite examination, including examination for *Cryptosporidia* organisms, *Isospora belli,* and *Blastocystis hominis* (ten stools), and electronmicroscopy for viral agents (five stools) were also done. Twenty-two case-patients and twenty-one asymptomatic controls were skin-tested for *Mycobacterium avium-intracellulare, Mycobacterium kansasii, Mycobacterium scrofulaceum, Mycobacterium tuberculosis, Candida albicans,* and *Trichophyton* species. Pathologists at CDC and the Baylor University Medical Center reviewed biopsy slides obtained from local hospitals.

Epidemiologic and Environmental Studies

To determine risk factors for chronic diarrheal illness in the community, we conducted a case-control study (case-control study 1) and a survey. Case-control study 1 included case-patients who were residents of Henderson County ($n = 23$) or the neighboring town of Kirkwood in Warren County ($n = 5$) and had consulted a physician for their diarrheal illness. One age- and neighborhood-matched control, with no diarrheal illness lasting longer than 1 week in the previous 3 months, was systematically selected for each case-patient. Truck drivers employed by an Iowa-based national retail company who frequently passed through Henderson County and had reported cases of chronic diarrheal illness to the health department were surveyed. In both the case-control study and the survey, data gathered included foods and beverages purchased (particularly foods and dairy products purchased from farms or produce stands), restaurants and food stores visited, domestic water supply used, exposure to animals, history of previous gastrointestinal illness or medication use, and illness among household contacts.

After these two studies implicated the restaurant, a second case-control study and a cohort study were done. In case-control study 2, 30 case-patients who named 65 meal-companion controls were analyzed in a matched design with a variable number of controls per case. The cohort study included all truck drivers identified in the survey who had eaten at the restaurant. Information asked included frequency, date and time of restaurant visits, specific food and beverages consumed in the restaurant, seating and parking preferences, restroom use, cigarette and alcohol use, history of gastrointestinal illnesses, and use of medications. All available restaurant employees were interviewed.

The restaurant was inspected by local and state sanitarians, and water quality reports from the restaurant and local community were reviewed. Water from the implicated well was analyzed for pesticides, herbicides, volatile organic compounds, and heavy metals. The Environmental Protection Agency cultured water for mycobacterial species (17). Viral cultures of water, human enteroviral gene probe of a water filter, and examination of water for ova and parasites were done at the University of Arizona (18-20).

The statistical analyses were done using the Wilcoxon rank sum test, the Fisher exact two-tail test, the McNemar test, and conditional logistic regression.

Results

Clinical Studies

Seventy-two case-patients were identified; 36 (50%) case-patients were male. The mean and median age of case-patients was 56 years (range, 9 to 83 years). Illness was characterized by sudden onset of severe watery diarrhea; in the initial week of illness, some case-patients described more than 20 stools per day (median, 12 stools/day). Urgency was reported by 100% of case-patients and was severe, often associated with fecal incontinence (65%). Weight loss was reported by 62% at 2 months (mean loss, 4.5 kg). Fever was uncommon. The frequency of symptoms is shown in Table 1.

Six months after the initial investigation, 62 (87%) of 71 case-patients who responded to the follow-up questionnaire continued to have diarrhea; 52 (73%) reported having diarrhea every day (mean duration of illness, 261 days). Frequency of bowel movements in those with daily diarrhea had decreased to a mean of six bowel movements per day. Complete resolution of diarrhea occurred in 7 case-patients (mean time until resolution, 183 days); these case-patients were younger than those who remained ill (median age, 37 compared with 56, $P = 0.01$).

A median incubation period of 10 days (range, 2 to 40 days) was estimated from 15 case-patients who visited the restaurant on only 1 day. Of 150 case-patient household members at risk for secondary transmission, none developed diarrhea. One Henderson County case-patient frequently visited her adult daughter in Iowa; that daughter developed chronic diarrhea 3 months after the mother's diarrhea onset; however, because sporadic cases of chronic diarrhea occur and because contact was difficult to quantify, it was not possible to definitely ascribe this case to secondary spread.

Of the 72 case-patients, 60 (83%) consulted a physician. Nine persons, eight of them women, were hospitalized; none died. Laboratory results are outlined in

Table 2. *Clinical Data for 67 Case-Patients in Henderson County, Illinois Compared with 78 Case-Patients in Brainerd, Minnesota with Chronic Diarrhea**

Laboratory Test	Henderson County	Brainerd	Results
	n(%)		*(n)*
Stool culture for *Shigella, Salmonella, Yersinia,* and *Campylobacter*	37(55)	43(55)	No pathogens (77), *Salmonella* (2), *Campylobacter jejuni* (1)
Stool for ova and parasites	39(58)	43(55)	No pathogens (79), *Blastocystis hominis* (2), *Giardia lamblia* (1)
Fecal leukocytes	9(13)	13(17)	None (4), few (16), moderate (1), many (1)
Barium enema	16(24)	31(40)	Normal (22), diverticuli (18), spasm (2), possible colitis (2)
Small-bowel series	8(12)	12(15)	Normal (17), duodenal diverticulum (1), rapid transit (1), gastric bypass (1)
Sigmoidoscopy	6 (9)	28(36)	Normal (29), proctitis (2), erythema (1), edema (1), punctate hemorrhages (1)
Colonoscopy	6 (9)	8(10)	Normal (5), nonspecific patchy erythema (4), inflammation (3), punctate hemorrhage (2)
Large-bowel biopsy	9(13)	9(12)	Normal (4), nonspecific mild, chronic inflammation (13), prominent lymphoid aggregates (1)
Small-bowel biopsy and aspirate	5 (7)	5 (6)	Normal (8), increased histiocytes (1), mild villous blunting (1), no *Giardia* (7)
Hematocrit	17(25)	19(24)	Normal (35), low (1)
Leukocyte cell count	18(26)	19(24)	Normal (33), low (2), high (2)

* Other tests performed on fewer than 10 case-patients (*n*) in Henderson County. All results were normal except where indicated: serum albumin (9; 3 low), thyroid function tests (6), vasoactive inhibitory peptide (2), gastrin (3; 1 high), 5-hydroxyindole acetic acid (3), serum B12 (3), serum folate (3), serum carotene (3; 1 high), qualitative fecal fats (2), quantitative fecal fats (3; 1 high).

Table 2; no consistent abnormalities to suggest inflammatory bowel disease or malabsorption were observed. Therapy included use of numerous antimicrobial and antidiarrheal agents (Table 3); none cured the illness. Opioid analogs provided subjective improvement in half of those receiving them.

All bacterial and mycobacterial stool cultures were negative for known pathogens, and no viral agents were seen on electronmicroscopic examination of stool specimens. *Giardia lamblia* was identified for one case-patient in the first of several stool specimens submitted for parasite examination. Diarrhea in this case-patient temporarily improved with metronidazole therapy but worsened several days later. A course of quinacrine hydrochloride also produced temporary improvement. Later examinations for stool parasites and a small-bowel aspirate did not show evidence of *Giardia* species although the case-patient continued to have five to six stools per day. Two case-patients had *B. hominis* noted in isolated stool samples. Both had continued symptoms despite later ova and parasite examinations that revealed no *B. hominis* organisms. Skin-test response to mycobacterial antigens was similar for case-patients and controls.

Twelve patients had sigmoidoscopy or colonoscopy; no gross abnormalities were seen. The mean interval from date of diarrhea onset to date of biopsy was 43 days. Local pathologists reported mild, nonspecific inflammatory changes in eight of the nine case-patients who had either colonic or rectal biopsies. All slides were reviewed at CDC and the Baylor University Medical Center, Dallas. The degree of inflammatory infiltrate was believed to fall within the normal range for colonic mucosa in all but two biopsies. Specifically, there was no evidence for either microscopic colitis or collagenous colitis.

Five case-patients had small-bowel biopsies. No parasitic infestation in any specimen and no significant abnormalities were noted by local pathologists. The four biopsy specimens reviewed at Baylor University Medical Center were believed to show a mild increase in plasma cells and eosinophils within the lamina propria. Similarly, the CDC pathologist reported increased plasma cells and eosinophils in three of five biopsies. In all cases, changes were concluded to be mild and nonspecific.

Epidemiologic and Environmental Studies

Attack rates among Henderson County residents were as follows: 0 in the group 0 to 4 years, 1.0/1000 in the group 5 to 17 years, 3.2/1000 in the group 18 to 64

Table 3. *Medications Used to Treat Chronic Diarrheal Illness in 67 Case-Patients in Henderson County, Illinois, 1987*

Medication*	Number Treated	Number Improved
	n	*n (%)*
metronidazole	19	3(18)
sulfonamide	14	2(15)
neomycin	10	2(17)
tetracycline	7	2(29)
bismuth salicylate	35	2 (6)
loperimide	32	18(64)
diphenoxylate	28	13(50)
Metamucil	15	1 (9)
cholestyramine	7	2(29)

* Other medications used by more than one case-patient include vancomycin (5), erythromycin (3), sulfasalazine (2), cimetidine (2), and Donnagel (3).

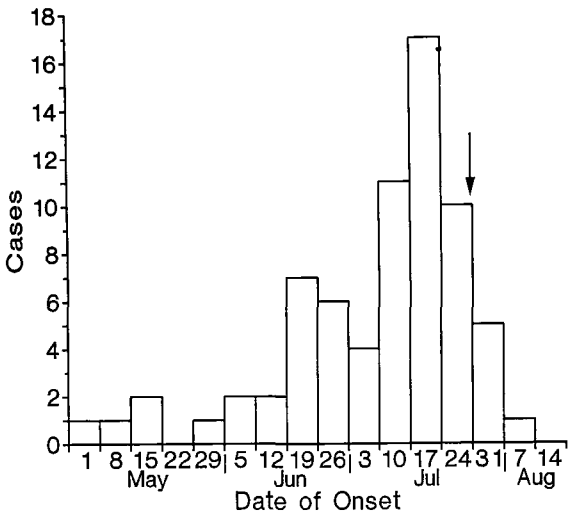

Figure 1. Number of cases of chronic diarrhea by week of onset, in Henderson County, Illinois, May through August 1987. The date of closure (7/31) of the implicated restaurant is indicated by the arrow.

years, and 9.2/1000 in group 65 years and older. The earliest onset dates were in early May, with a peak in mid-July, and a precipitous drop in the beginning of August (Figure 1). The last case-patient had an onset on 7 August, 1 week after the restaurant was closed. Thirty (42%) of the case-patients resided in Henderson County.

Eating at the restaurant in the 4 weeks before onset of illness was strongly associated with illness in case-control study 1. Twenty-five (89%) of twenty-eight case-patients visited the restaurant, and 6 (21%) of 28 neighborhood controls visited the restaurant (odds ratio = 19.0, $P < 0.001$).

Sixty-three (79%) of eighty truck drivers responded to the survey questionnaire and nine cases (14%) were identified. Three truckers described diarrheal illness but did not supply enough information to categorize them as case-patients and they were excluded from further analysis. One trucker did not respond to questions concerning restaurants. Nine (20%) of forty-five truckers who ate at the restaurant and none of 14 truckers who did not eat at the restaurant were case-patients (relative risk = indeterminate, $P = 0.1$, Fisher exact two-tail test).

The results of case-control study 2 (study of restaurant-associated risk factors for illness) are outlined in Table 4. Case-patients were older than controls (median age of 60.5 years for case-patients and 52 years for controls, $P = 0.02$), and visited the restaurant more frequently than controls (median visits in June and July, four for case-patients and two for controls, $P = 0.5$). The only exposure that increased the risk of developing illness was drinking water at the restaurant. Twenty-nine (97%) of thirty case-patients reported drinking water at the restaurant, compared with 40 (71%) of 56 controls ($P = 0.04$; 9 controls who could not recall whether they drank water were excluded from analysis). Water remained a risk factor for illness when controlled for number of visits to the restaurant ($P = 0.03$) and age ($P = 0.06$). Drinking coffee prepared with water heated to 65 °C did not increase the risk of illness (odds ratio = 1.0). The restaurant did not serve unpasteurized milk products.

Total restaurant water consumption was estimated by multiplying the average number of glasses consumed per visit times the estimated number of visits in the 3-month period from May to July. Case-patients drank more water at the restaurant than did matched controls (median, 4.0 glasses compared with 1.8 glasses; $P = 0.3$). When the one case-patient and 16 controls who drank no water were excluded from analysis, this difference in total water consumption became less pronounced (median, 3.0 glasses compared with 2.0 glasses). No trend to suggest a dose-response was observed even when controlled for age.

Case-patients and controls were similar in their use of antibiotics (Table 4), antacids, and histamine blockers.

Twenty-four (53%) of the forty-five truckers who had visited the restaurant participated in the truck-driver cohort study. The chronic diarrhea attack rate was higher among the older age group (Table 5). A high frequency of restaurant visits also increased risk

Table 4. *Risk Factors for Diarrheal Illness in the Restaurant Matched Case-Control Study, Henderson County, Illinois*

Matched Risk Factor	Case-Patients	Controls	Odds Ratio	95% Confidence Interval	*P* Value*
Univariate					
Female	50%	57%	0.8	0.3 to 1.9	0.6
Mean age	60.5y	52.0y	NA†	NA	0.02
Median number of restaurant visits, June to July	4	2	NA	NA	0.5
Drank water	97%	71%	9.3	1.2 to 73.8	0.04
Drank coffee	63%	63%	1.0	0.4 to 2.6	1.0
Previous antibiotic use	7%	10%	1.5	0.3 to 8.4	0.6
Multivariate					
Drank water			10.2	1.3 to 82.0	0.03
Visit frequency					0.3
Drank water			7.5	0.9 to 61.5	0.06
Age					0.05
Drank water			10.0	1.2 to 81.2	0.03
Drank coffee			1.3	0.3 to 3.7	0.7

* Statistics done using conditional logistic regression.
† Not applicable.

Table 5. *Risk Factor for Chronic Diarrhea Cohort Study of 24 Truck Drivers in Henderson County, Illinois, 1987*

Variable	Total	Number of Case-Patients	Attack Rate*	Relative Risk	95% Confidence Interval	*P* Value†
Age, *y*						
≤ 40	11	1	9.1	1.0		
> 40	13	8	61.5	6.8	1.0 to 46.0	0.01
Visits to the restaurant, *n*						
< 15	12	1	8.3	1.0		
≥ 15	12	8	66.7	8.0	1.2 to 54.5	0.009
Glasses of water drunk,‡ *n*						
1-10	8	1	12.5	1.0		
11-30	8	3	37.5	3.0	0.4 to 23.1	0.6
> 30	7	4	57.1	4.6	0.7 to 31.9	0.1
						0.07§

* Attack rate in case-patients per 100 persons exposed.
† Fisher exact test, two-tailed.
‡ Excludes one person who did not drink water.
§ Chi-square due to linear trend.

of illness as did drinking a greater total volume of water (Table 5).

The restaurant employed 11 people, 8 of whom were interviewed. Three employees met the case-patient definition. Two additional employees, including the cook-manager, described prolonged diarrheal illness but did not meet the case-patient definition. The cook-manager had *G. lamblia* in a stool sample and reported cure with metronidazole therapy. The other employee described having two to three loose stools per day only intermittently over the summer months.

Henderson County rests on limestone through which water drains from east to west into the Mississippi River. Although there are scattered beef cattle, there are no dairies within the county. Municipal water is available within the county's towns, but county residents who live in more rural areas rely on either private shallow sandpoint wells or deep wells that penetrate into the limestone aquifer.

The restaurant obtained untreated water from a 4-inch drilled well, 125 feet deep, which also supplied a neighboring, vacant house. Routine water samples collected between September 1981 and May 1987 showed satisfactory results for total coliforms (15 samples) and total nitrate (16 samples). In June 1987, three power failures and two mild earthquakes occurred at or near the well. The restaurant continued using water during these power failures. On 30 July, 1 day before the restaurant was closed, a water sample collected from the restaurant's combination soft drink and water dispenser showed a high total coliform count (> 16/100 mL, tube dilution method). On 1 August, the well was hyperchlorinated.

The well had been inspected in April 1987, and the owners were put under notice to correct several construction deficiencies. At a May reinspection, deficiencies remained that could have permitted water from contaminated sources to siphon back into the well, particularly from within the vacated house and from abandoned, blind pipes that branched from the well into a surrounding field. In the 2 months after chlorination, 2 of 20 water samples taken from the well or its attached plumbing showed a mild elevation of fecal coliforms. One sample, collected on 9 September from the vacant house, revealed a high fecal coliform count (2300 organisms/100 mL of water). Several nearby wells of varying depths tested satisfactorily.

Microscopic examination of water collected on 9 September revealed no parasites. A water filter was nonspecifically positive by gene probe for a human enterovirus; however, viral cultures of the water grew no organisms. No pesticides, herbicides, heavy metals, or volatile organic agents were detected from well water collected on 9 September. The restaurant owner drilled a new well, replaced the restaurant's plumbing system, and reopened on 8 October. No subsequent illness has been reported.

Discussion

This is the first reported outbreak of chronic diarrhea associated with drinking untreated well water. The dramatically severe symptoms in this and the two previously described outbreaks suggest a distinct illness with a common pathophysiologic mechanism and causative agent. Because an environmental exposure appears to cause illness, a standard case definition is needed so that responsible vehicles can be identified and transmission interrupted. We suggest a case definition for "Brainerd diarrhea" that includes the following criteria: nonbloody diarrhea lasting longer than 1 month with negative stool examination for known bacterial and parasitic pathogens; acute onset of illness; fecal urgency; absence of fever; eight or more stools per day throughout the first week of illness; normal stool fecal fat or D-xylose test, or both; and normal or mild, chronic inflammatory changes on colon biopsy. The proposed case definition, though untested, would distinguish this chronic diarrheal syndrome from mild diarrheal illness that may be heterogeneous in cause and from inflammatory bowel diseases, malabsorptive diseases, and collagenous or microscopic colitis.

The causative agent of outbreak chronic diarrhea remains obscure. Heating water to 65 °C may have prevented transmission of illness, however, suggesting either a heat-labile toxin or a microorganism. In the Minnesota outbreak, a possible case of person-to-per-

son transmission suggested an infectious pathogen. We were unable to confirm this finding in Henderson County. *Blastocystis hominis* organisms were found in the stools of two case-patients; each of these case-patients later had negative stool examinations despite continuing symptoms. Furthermore, the clinical picture ascribed to *Blastocystis* infection—recurrent abdominal cramps, fatigue, and anorexia with only mild diarrhea—is not consistent with the outbreak illness (21, 22). We believe *B. hominis* infection to be an incidental finding in these two case-patients, and no other known pathogens were identified.

Microscopic colitis and collagenous colitis, two newly defined pathologic entities, both cause watery, urgent, chronic diarrhea (4-8). Although these symptoms are compatible with the outbreak illness, the mild pathologic abnormalities noted on Henderson County biopsy specimens are not consistent with either microscopic or collagenous colitis. In microscopic colitis, mucosal goblet cell depletion, excess neutrophils, round cells within the lamina propria, and penetration of neutrophils into the surface epithelium are typically seen (9). In collagenous colitis, the subepithelial collagen layer is thickened, and lymphocytes and plasma cells infiltrate the lamina propria (7, 23). Neither the acute inflammation of microscopic colitis nor the collagen banding of collagenous colitis was evident in the Henderson County biopsies. However, the time from onset of illness to colon biopsy was comparatively brief (mean, 43 days). Collagenous colitis and microscopic colitis are generally diagnosed histopathologically after more than 1 year of symptoms. It is conceivable that the three clinical entities represent different stages of the same disease. In some studies, progression from microscopic colitis to collagenous colitis has been demonstrated (24, 25). Outbreak diarrhea may be the *forme fruste*. Sequential colonic biopsies of Henderson County patients are planned to assess this possible association.

Subtle changes in small-bowel mucosal biopsies were also present; however, clinical studies by local physicians do not evince malabsorption. Three patients from the Minnesota outbreak had abnormal small-bowel motility, but again, abnormalities were inconstant and nonspecific (26). Understanding these mild changes would be enhanced by follow-up studies.

Although recall of water consumption by case-patients and controls was subject to considerable bias, the association between the restaurant's water and illness was both strong and plausible. Unlike food items that had rapid turnover in the restaurant, well-water usage continued uninterrupted throughout the outbreak period. Some restaurant patrons reported that the water appeared filmy or foamy, or smelled foul. Inspections of the restaurant and the well revealed plumbing deficiencies that could have permitted water contamination; a sample taken from the water dispenser 1 day before the restaurant closed was contaminated with coliforms. Several points along the plumbing system (for example, filters, the water softener, or the combination water-soft-drink dispenser) could have been a source for continuous or sporadic water contamination. Furthermore, the restaurant continued to use water during power failures, enhancing the opportunity of contamination by back-siphonage. The unlikely possibility remains however, that water directly from the limestone aquifer could have been responsible.

A substantial proportion of sporadic, chronic diarrheal illness may be caused by exposure to an as yet unidentified environmental agent. Because no diagnostic test for this illness exists, it is important that both primary-care physicians and specialists recognize the syndrome clinically and inquire about case clusters and possible exposures to untreated water or unpasteurized milk. Improved national awareness, early case recognition, and continued clinical and epidemiologic investigation of both sporadic and outbreak-related chronic diarrhea are fundamental to further definition of this debilitating disease.

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References

1. **Osterholm MT, MacDonald KL, White KE, et al.** An outbreak of a newly recognized chronic diarrhea syndrome associated with raw milk consumption. *JAMA.* 1986;**256**:484-90.
2. **Martin DL, Hoberman LJ.** A point source outbreak of chronic diarrhea in Texas: no known exposure to raw milk [Letter]. *JAMA.* 1986;**256**:469.
3. **Read NW, Krejs GJ, Read MG, Santa Ana CA, Morawski SG, Fordtran JS.** Chronic diarrhea of unknown origin. *Gastroenterology.* 1980;**78**:264-71.
4. **Rams H, Rogers AI, Ghandur-Mnaymneh L.** Collagenous colitis. *Ann Intern Med.* 1987;**106**:108-13.
5. **Wang KK, Perrault J, Carpenter HA, Schroeder KW, Tremaine WJ.** Collagenous colitis: a clinicopathologic correlation. *Mayo Clin Proc.* 1987;**62**:665-71.
6. **Kingham JG, Levison DA, Morson BC, Dawson AM.** Collagenous colitis. *Gut.* 1986;**27**:570-7.
7. **Jessurun J, Yardley JH, Giardiello FM, Hamilton SR, Bayless TM.** Chronic colitis with thickening of the subepithelial collagen layer. *Hum Pathol.* 1987;**18**:839-48.
8. **Kingham JG, Levison DA, Ball J, Dawson AM.** Microscopic colitis—a cause of chronic watery diarrhoea. *Br Med J.* 1982;**285**:1601-4.
9. **Bo-Linn GW, Vendrell DD, Lee E, Fordtran JS.** An evaluation of the significance of microscopic colitis in patients with chronic diarrhea. *J Clin Invest.* 1985;**75**:1559-69.
10. **Svennerholm AM, Holmgren J.** Identification of *Escherichia coli* heat-labile enterotoxin by means of a ganglioside immunosorbent assay (G_{M1}-ELISA) procedure. *Curr Microbiol.* 1978;**1**:19-23.
11. **Lockwood DE, Robertson DC.** Development of a competitive enzyme-linked immunosorbent assay (ELISA) for *Escherichia coli* heat-stable enterotoxin (STa). *J Immunol Methods.* 1984;**75**:295-307.

12. **Nataro JP, Baldini MM, Kaper JB, Black RE, Bravo N, Levine MM.** Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. *J Infect Dis.* 1985;**152**:560-5.
13. **Moseley SL, Clausen CR, Smith AL.** A new bacterial adhesin associated with enteritis in infants [Abstract]. In: *25th Interscience Conference on Antimicrobial Agents and Chemotherapy.* Washington D.C.: American Society for Microbiology; 1985:1128.
14. **Newland JW, Neill RJ.** DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. *J Clin Microbiol.* 1988; **26**:1292-7.
15. **Venkatesan M, Buysse JM, Vandendries E, Kopecko DJ.** Development and testing of invasion-associated DNA probes for detection of *Shigella* spp. and enteroinvasive *Escherichia coli. J Clin Microbiol.* 1988;**26**:261-6.
16. **National Veterinary Service Laboratories.** *Laboratory Methods in Veterinary Mycobacteriology for the Isolation and Identification of Mycobacteria.* Ames, Iowa: U.S. Department of Agriculture; 1985.
17. **du Moulin GC, Stottmeier KD.** Use of cetylpyridinium chloride in the decontamination of water for culture of mycobacteria. *Appl Environ Microbiol.* 1978;**36**:771-3.
18. **Gerba CP, Margolin A, Trumper B, Rose JB, Zhang C.** Low cost rapid methods for enterovirus detection in water. In: *Water Quality Technology Conference. Advances in Water Analysis and Treatment.* Denver: American Water Works Association; 1987:417-24.
19. **Rose JB, Madore MS, Riggs JL, Gerba CP.** Detection of cryptosporidia and giardia in environmental waters. In: *Water Quality Technology Conference. Advances in Water Analysis and Treatment.* Denver: American Water Works Association; 1987:1025-41.
20. **Musial CE, Arrowood MA, Sterling CR, Gerba CP.** Detection of *Cryptosporidia* in water using polypropylene cartridge filters. *Appl Environ Microbiol.* 1987;**53**:687-92.
21. **Sheehan DJ, Raucher BG, McKitrick JC.** Association of *Blastocystis hominis* with signs and symptoms of human disease. *J Clin Microbiol.* 1986;**24**:548-50.
22. **Markell EK, Udkow MP.** *Blastocystis hominis:* pathogen or fellow traveler? *Am J Trop Med Hyg.* 1986;**35**:1023-6.
23. **Giardiello FM, Bayless TM, Jessurun J, Hamilton SR, Yardley JH.** Collagenous colitis: physiologic and histopathologic studies in seven patients. *Ann Intern Med.* 1987;**106**:46-9.
24. **Teglbjaerg PS, Thaysen EH, Jensen HH.** Development of collagenous colitis in sequential biopsy specimens. *Gastroenterology.* 1984;**87**:703-9.
25. **Jessurun J, Yardley JH, Lee EL, Vendrell DD, Schiller LR, Fordtran JS.** Microscopic and collagenous colitis: different names for the same condition? [Letter]. *Gastroenterology.* 1986;**91**:1583-4.
26. **Kellow J, Phillips S, Miller L, Osterholm M, MacDonald KL.** Abnormalities of motility and absorption in an outbreak of chronic diarrhea [Abstract]. *Gastroenterology.* 1985;**88**:1442.

AMERICAN JOURNAL OF Public Health

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Epidemic Giardiasis Caused by a Contaminated Public Water Supply

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Abstract: In the period November 1, 1985 to January 31, 1986, 703 cases of giardiasis were reported in Pittsfield, Massachusetts (population 50,265). The community obtained its water from two main reservoirs (A and B) and an auxiliary reservoir (C). Potable water was chlorinated but not filtered. The incidence of illness peaked approximately two weeks after the city began obtaining a major portion of its water from reservoir C, which had not been used for three years. The attack rate of giardiasis for residents of areas supplied by reservoir C was 14.3/1000, compared with 7.0/1000 in areas that received no water from reservoir C. A case-control study showed that persons with giardiasis were more likely to be older and to have drunk more municipal water than household controls. A community telephone survey indicated that over 3,800 people could have had diarrhea that might have been caused by *Giardia*, and 95 per cent of households were either using alternate sources of drinking water or boiling municipal water. Environmental studies identified *Giardia* cysts in the water of reservoir C. Cysts were also detected in the two other reservoirs supplying the city, but at lower concentrations. This investigation highlights the risk of giardiasis associated with unfiltered surface water systems. (*Am J Public Health* 1988; 78:139–143.)

Introduction

Waterborne giardiasis is becoming an important public health problem in the United States: during the period from 1965–84, 90 outbreaks and 23,776 cases of giardiasis were reported. Sixty-nine per cent of outbreaks and 74 per cent of cases related to contamination of public water supplies, the majority of which were surface water systems with inadequate filtration or chlorination.[1] We describe one of the largest waterborne outbreaks of giardiasis ever reported in the United States and highlight the risk of giardiasis in communities that are supplied by unfiltered surface water systems.

Background

The outbreak occurred in the western Massachusetts city of Pittsfield (population, 50,265, 1985 census). The problem was first recognized during the first week of December 1985, when the health department of Pittsfield received 70 reports of laboratory-confirmed giardiasis. Only 53 positive stool samples for *Giardia* had been submitted during the entire one-year prior to November 1, 1985. Interviews of the 70 case-patients revealed that they had negative stool cultures for bacterial enteric pathogens, no common personal contact, and they were widely distributed throughout the city. This information, coupled with the knowledge that a new reservoir had been added to the community water supply in November, led city and state health officials to suspect a waterborne outbreak of giardiasis.

Water Supply

Prior to November 1985, the community obtained its drinking water from two surface reservoirs, A and B. Water was chlorinated but not filtered. However, during November 1985, alterations were made to the water system to facilitate the construction of a filtration system for water originating from reservoir A. Specifically, on November 5, an auxiliary reservoir C was brought on line for the first time in over three years to replace water from reservoir A which was to be phased out while the filtration system was being installed. This was expected to be a temporary yet necessary step in order to meet the usual demand for water, as installation of filtration equipment for reservoir A required decreasing the flow of reservoir A water into the city's water system during the construction period. On November 14, as construction proceeded, the flow from reservoir C was increased and the flow from reservoir A was correspondingly increased. On this day, the water department received complaints of turbid water from many areas of the city; the increased turbidity was attributed to reversals in water flow which suspended sediment in the water mains. After approximately two days, the turbidity resolved spontaneously. Reservoir C supplied water to the city until December 21, when it was replaced by reservoir A.

Methods

Epidemiologic Investigation

Daily reports of positive *Giardia* isolates were obtained from the two clinical laboratories in the region. The investigators attempted to reach every reported case-patient by telephone; patients were asked to report demographic information and the onset date of symptoms. Local physicians were sent letters encouraging them to obtain stool analyses from patients with symptoms clinically consistent with giardiasis, and were urged to report positive cases to the local health department.

Laboratory analysis of stool samples were performed locally by the major clinical laboratory in the area. Stool samples were cultured for *Salmonella*, *Shigella*, and *Campylobacter*. Analysis for ova and parasites was performed using standard direct saline and formalin-ethyl acetate methods.[2]

A confirmed case of outbreak-associated giardiasis was defined as a Pittsfield resident with a stool sample positive for *Giardia lamblia* submitted after November 1, 1985. A suspect case of giardiasis was anyone interviewed in the community surveys who reported having had diarrhea (≥3 loose stools per 24-hour period) of undetermined etiology of ≥5 days duration with onset since November 1, 1985.

Information obtained from the city water engineer con-

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Editor's Note: See also related editorial p 123 this issue.

cerning placement of values in the municipal water system was used to construct a water distribution map depicting the reservoir origin of drinking water and the distribution pattern throughout the city between November 1, 1985 and January 31, 1986 (Figure 1). We estimated the population in each of these regions using census tract data obtained in March 1985; populations in areas that were included in more than one census tract data were divided in proportion to the length of city streets in each area. Attack rates were then determined for each water distribution area by counting the number of reported case-patients residing in each region divided by the estimated population.

Case-Household Telephone Survey

Questionnaires were administered by telephone to each of the first 139 reported case-patients during the first week of the investigation (December 13–19); each of these case-patients was asked to supply information about all household members. Whenever possible, all household members were interviewed individually. They were asked to describe their principal source of drinking water and the number of glasses of beverages containing both unboiled and boiled municipal water they customarily drank per day, as well as any gastrointestinal symptoms they may have experienced.

A case-control study was performed using the survey data to identify risk factors for giardiasis. Cases were defined as persons with laboratory-confirmed giardiasis; controls were defined as persons who did not have positive stool samples and had no diarrhea. Cases and controls were matched within households, and conditional logistic regression[3] was used to determine whether age and water consumption were independently associated with giardiasis.

Case Telephone Survey

Questionnaires similar to those used in the case-household survey were administered to the next 100 reported case-patients. Additional questions were included for these 100 persons specifically describing their consumption of municipal water at home and away from home. Information about household members was not obtained.

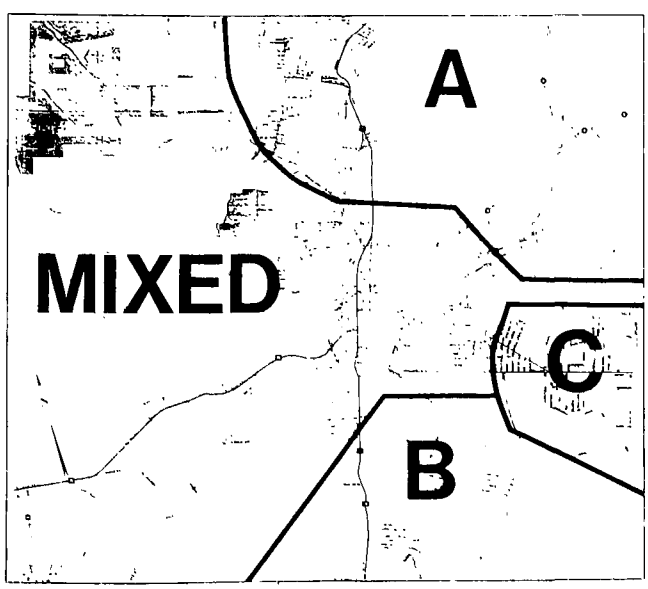


FIGURE 1—Water Distribution by Reservoir Source, Pittsfield, Massachusetts, November 1 to December 21, 1985

Community Telephone Survey

A community telephone survey of 220 households randomly selected from the telephone directory was conducted on January 16, 1986. The purpose of the survey was to determine whether laboratory reports of positive stool samples were true indicators of the occurrence of disease in the community. By this time, physicians may have been treating an increasing number of symptomatic patients empirically without having obtained stool samples because of the widespread publicity associating diarrhea with giardiasis. Each household member was to be interviewed concerning water consumption, gastrointestinal symptoms, diagnosis, and treatment. Heads of households were also asked about compliance with control measures. The systematic sampling technique was performed using methods described by Kish,[4] and 95% confidence intervals (CI) of estimated numbers of cases were determined using a method described by Mendenhall.[5]

Environmental Investigation

We reviewed all available water quality testing results indicating turbidity, coliform, and chlorine levels since January 1, 1985. Personnel from city and state environmental engineering agencies inspected the water distribution system for cross-connections with sewage lines.

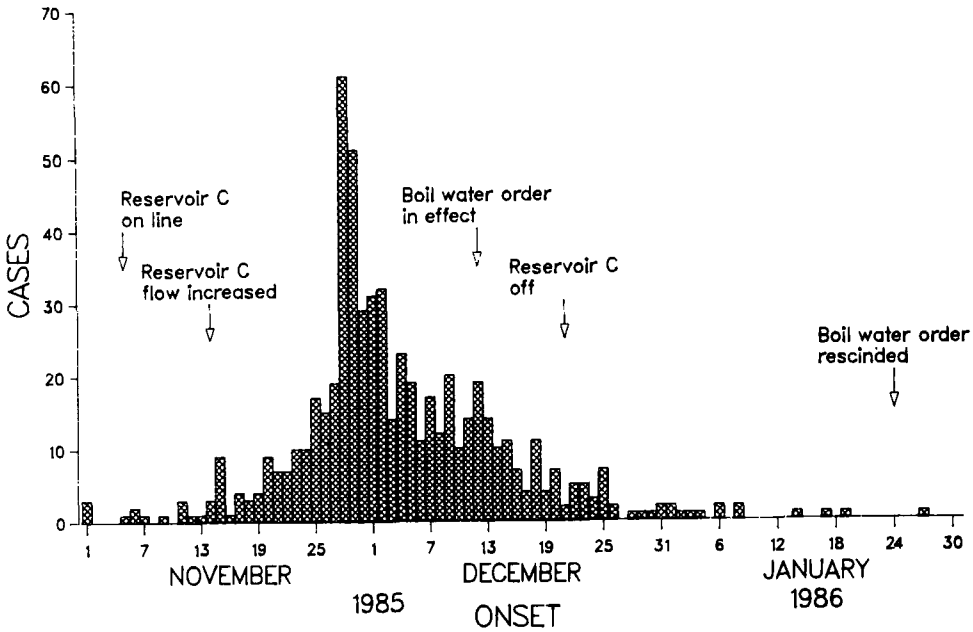
Chlorinated water from reservoir C immediately distal to the chlorination point was sampled for *Giardia* cysts on December 12; untreated water in all three reservoirs was sampled on December 16; additional samples were obtained from three sites in the distal portion of the distribution system on four occasions in late December and early January. Sampling for *Giardia* cysts was performed using volume-sampling orlon yarn filters supplied by the US Environmental Protection Agency (EPA).[6] Analysis of water samples for *Giardia* was also performed by the EPA using an immunofluorescent antibody technique.[7] *Giardia* cysts extracted from the water samples obtained from each of the three reservoirs were tested for infectivity by inoculation in gerbils.[8] *Giardia* trophozoites obtained from these animals were then examined at the University of Minnesota by scanning electron microscopy for morphological characteristics in order to determine whether they were from human or animal origin.

Inspectors surveyed the watershed areas of the three reservoirs for signs of sewage contamination and beaver activity in December 1985; beavers obtained during the survey were examined for *Giardia*. During the summer of 1986, muskrats were obtained from the watershed areas of all three reservoirs and examined for infection with *Giardia*.

Eleven outbreak-related human fecal samples were sent to the University of Minnesota for use in a cross-species transmission experiment. Beavers and muskrats were administered *Giardia* cysts from these samples and examined for infection. *Giardia* trophozoites obtained from these animals were also studied for morphological characteristics.

Results

From November 1, 1985 to January 31, 1986, 703 persons were identified with positive stool samples for *Giardia*. During this same period, approximately 2,600 persons submitted stool samples, yielding a *Giardia*-positive rate of 27 per cent. Onset dates of illness were obtained from 604 (86 per cent) of the 703 confirmed case-patients, and residential address was confirmed for 635 persons (90 per cent). The greatest number of persons reported onset of symptoms on



*604 stool-positive cases. Date of onset unknown for 99 cases.

FIGURE 2—Laboratory-confirmed Cases of Giardiasis, by Date of Onset, Pittsfield, Massachusetts, November 1, 1985 to January 31, 1986

November 28, two weeks after the flow from reservoir C was increased and the episode of turbid water occurred (Figure 2). When attack rates of laboratory-confirmed giardiasis were calculated by place of residence, the highest attack rates were found for areas of the city served by reservoir C (Table 1).

Case-Household and Case Telephone Surveys

In the case-household survey of 139 households, information was obtained about all 437 household members. Eight additional persons with positive stool samples, who had not yet been reported, were identified, yielding 147 confirmed case-patients. Of the next 100 case-patients who were interviewed in the case telephone survey, seven had been interviewed before in the case-household survey; thus, a total of 240 confirmed case-patients were interviewed in detail concerning their symptoms. Among these 240, 236 (98 per cent) had diarrhea. The mean duration of diarrhea was 11.3 days, with a range of 1–34 days. Additional gastrointestinal symptoms were frequent (Table 2).

Among the 139 households interviewed, we identified 147 persons with laboratory-confirmed giardiasis (cases) and 239 persons who had neither diarrhea nor positive stool samples for *Giardia* (controls). Seventy-three per cent (108/147) of cases reported drinking two or more glasses of municipal water per day, compared to 36 per cent (86/239) of controls (OR = 4.9 95% CI = 3.1–8.0). Eighty-eight per cent (130/147) of cases were age 20 years or older, compared with 52 per cent (124/239) of controls (OR = 7.1 95% CI = 3.9–13.3). Conditional logistic regression analysis, used to match cases and controls by household and to control for age and water consumption, indicated that drinking two or more glasses of unboiled municipal water per day and being age 20 years or older were independently associated with giardiasis [OR (water) = 9.2 95% CI = 4.2–19.9; OR (age) = 3.3 95% CI = 1.7–6.2].

TABLE 1—Attack Rates by Residential Water Source, Laboratory-confirmed Cases of Giardiasis, Pittsfield, Massachusetts, November 1, 1985 to January 31, 1986

Water Source	Population	No. Cases	Attack Rate (per 1000)
Reservoir A	9405	68	7.2
Reservoir B	2309	14	6.1
Reservoir C	4200	126	30.0
Mixed (A, B, and C)	34351	427	12.4
TOTAL	50265	635	12.6
Residential water from reservoir C	**Population**	**No. Cases**	**Attack Rate (per 1000)**
Yes	38551	553	14.3
No	11714	82	7.0
TOTAL	50265	635	12.6

TABLE 2—Frequency of Symptoms, Laboratory-confirmed Cases of Giardiasis, Case-Household and Case-Telephone Surveys, Pittsfield, Massachusetts, November–December 1985

Symptom	Persons with Symptom (%)
Diarrhea (any duration)	236 (98)
(≥ 5 days)	190 (79)
Flatulence	214 (89)
Fatigue	207 (86)
Abdominal cramps	204 (85)
Loss of appetite	197 (82)
Bloating	180 (75)
Nausea	178 (74)
Weight loss	165 (69)
Vomiting	87 (36)
Fever (undocumented)	37 (15)
Bloody diarrhea	7 (3)
(N=240)	

Among 100 case-patients interviewed concerning the number of glasses of unboiled municipal water consumed at home or away from home, 74 reported that all or most of their water consumption occurred at home. They reported drinking a mean of 3.2 glasses per day at home, and 0.9 glasses per day away from home.

Community Telephone Survey

Of the 220 households selected, 200 were contacted; we obtained information on all 527 persons living in these households. Fourteen persons (2.6 per cent) had positive stool samples for *Giardia* since November 1 (confirmed cases); all of these persons had had diarrhea for ≥ 5 days before receiving treatment. Twenty-six persons (5 per cent) had diarrhea of undetermined etiology with ≥ 5 days duration (suspect cases). Projections from this survey indicated that between November 1, 1985 and January 15, 1986, 1,335 people (95% CI = 598–2072) had confirmed giardiasis and 2,480 additional persons (95% CI = 1426–3534) were suspect cases. The onset date of illness in suspect cases resembled that of the confirmed cases, suggesting that many of these suspect cases could have been outbreak-associated. Relatively few cases were reported with onset in January, which was also consistent with reports from laboratory-confirmed cases.

Of the 200 households interviewed, 189 (95 per cent) reported continuous compliance with a boil-water order that had been issued on December 12; 78 (39 per cent) were boiling municipal water, and 111 (56 per cent) were either using commercial bottled water or were obtaining water from local springs or wells. All respondents indicated awareness of the outbreak and the boil-water order.

Environmental Investigation

Daily records of free residual chlorine levels in water entering the distribution system immediately distal to the chlorination point indicated that chlorine levels in water from reservoir C were between 0 and 0.5 mg/l during the entire month of November due to a malfunction in the chlorinating machinery which was not successfully repaired until November 30. During December, chlorine levels in the distribution system water from reservoir C averaged 2 mg/l with approximately 15 minutes minimum contact time before water reached the first households. Chlorine levels in water entering the distribution system from reservoirs A and B immediately distal to the chlorination point during both months averaged 3 mg/l and 2 mg/l, respectively, with 15-minute minimum contact times. Water temperature in all three reservoirs was approximately 40°F, measured turbidity levels were within acceptable limits, and pH ranged from 7.0 to 7.4.

During October 1985, all bacterial cultures of water samples from various points in the distribution system contained levels of coliform bacteria below the acceptable limit of 4 colony forming units (CFU)/100 ml; no bacteria were detectable in 71 of 80 samples. However, on November 19, five days after the episode of turbid water (observed but not measured), five of 17 samples had > 5 CFU/100 ml. All of these five samples were taken from areas of the city that were at least partially supplied by water from reservoir C. Sampling was performed daily for the next seven days; after two days, all samples were negative for coliform bacteria except for a single site located in the region served exclusively by reservoir C, which continued yielding elevated coliform counts (8–41 CFU per 100 ml) until December 1, when the defective chlorinator was repaired.

Microscopic examination of water samples revealed that chlorinated water from reservoir C obtained immediately distal to the chlorination point contained 80 *Giardia* cysts/100 gallons; untreated water obtained directly from reservoir C contained 28 *Giardia* cysts/100 gallons. The samples obtained on December 16 directly from untreated water in reservoirs A and B contained seven cysts and nine cysts/100 gallons, respectively. Subsequent water samples taken from distal points in the distribution system in December and January yielded no *Giardia* cysts. *G. lamblia* cysts and trophozoites were detected in the laboratory animals inoculated with the extract from untreated reservoir C water; animals receiving portions of water samples obtained from reservoirs A and B were negative. Morphological studies suggested that the trophozoites obtained from reservoir C did not resemble those of beaver origin, but instead more resembled trophozoites found in humans. *Giardia* cysts that were isolated from the outbreak-related human fecal samples and inoculated in beavers and muskrats produced infection in both types of animals and also resembled *Giardia* typically of human origin.

The original source of contamination for reservoir C was not identified. Inspectors found no evidence of sewage contamination in any of the water mains or in any of the watershed areas of the three reservoirs. However, they encountered signs of human activity (graffiti and empty beverage containers) near the shoreline of reservoir C in officially restricted areas; they also noted many signs of recent beaver activity (fresh decorticated wood cuts) near reservoir C, and near a small pond that ultimately drained into reservoir B, but no evidence of beavers near reservoir A. Nine beavers were found during the December 1985 survey of the three watershed areas; one of three beavers found near reservoir C yielded stool samples positive for *Giardia*, compared to none of six beavers found in the watershed area of reservoir B. All of seven muskrats obtained during summer 1986 from watershed areas of all three reservoirs were infected with *Giardia*. Thus, the water and animal studies suggested that beavers and muskrats in the area contributed to contamination of the water supply and may have originally been infected from a human source, but they did not determine whether the reservoir water had also been directly contaminated by human sewage.

Control measures for the outbreak included the following:

- A boil-water order was issued on December 12, 1985. Pittsfield residents were asked to either boil municipal water or use alternative sources of drinking water.
- Reservoir C water was removed from the municipal water supply on December 21, 1985.
- Water in storage tanks and in the distribution system was hyperchlorinated and flushed in order to kill any remaining *Giardia* cysts that may have settled in the water mains. On December 21, free residual chlorine levels were increased to 3 mg/l, and minimum contact times with chlorine were increased to one hour. The entire distribution system was then flushed over a nine-day period.
- The boil-water order was removed after the following conditions were met: a) laboratory reports showed no evidence of significant increase in new illness after two incubation periods (four weeks) following completion of the hyperchlorination and flushing; b) results of the second community survey over the same two incubation periods were consistent with the data from confirmed cases, showing no evidence of continuing infection; c) adequate chlorination and contact times were maintained; d) water

sampling during January in the distribution system revealed no *Giardia* cysts.

- Continued weekly reporting of laboratory-confirmed cases of giardiasis was required for at least one month after removing the boil-water order.

The boil-water order was rescinded on January 24, 1986, six weeks after it was issued. As of April 1, 1986, only one case of giardiasis had been reported with onset of symptoms after late January.

Discussion

It is difficult to maintain appropriate concentrations of chlorine that prevent waterborne giardiasis; thus, communities should not rely upon chlorination alone to protect public water supplies. Proposed additions or alterations to municipal water supplies which lack filtration should be thoroughly evaluated to ensure that contamination levels do not exceed the capacity of the chlorination system. Untreated water should be examined for the presence of fecal coliforms, turbidity and, if possible, *Giardia* cysts. This outbreak was facilitated by a malfunction in the chlorinating machinery which left very low levels of chlorine in water from reservoir C, but even if chlorine levels had been maintained at 2 mg/l for 15 minutes as intended, viable *Giardia* cysts might still have been present; water at 40°F and pH 7.0 probably requires more than 30 minutes of contact with 2 mg/l of chlorine to adequately kill *Gardia* cysts.[9]

The dramatically increased incidence of illness noted during the last few days of November suggests that a relatively high dose of *Giardia* cysts was present in drinking water during a short period of time. Assuming a mean incubation period of two weeks, as is commonly described, the time course of infection implicates the episode of turbid water on November 14, 1985 as being potentially responsible for the increased number of cases. *Giardia* cysts that had settled to the bottom of water mains could have been resuspended by the increased turbulence in water flow. The result could have been a bolus of higher concentrations of *Giardia* cysts causing more infections. Additionally, the increased turbidity probably reduced the effect of chlorine which was already at inadequate levels. The increased concentrations of bacteria in water samples during the week following the episode of turbid water could have occurred as a result of inadequate chlorination.

The validity of calculating attack rates by place of residence to implicate a specific water source depends upon the assumption that most water consumption occurs at home; our survey supports this assumption. John Snow, the famous British epidemiologist, used this technique effectively to implicate the Southwark and Vauxhall Company as the source of contaminated water in his classic cholera investigations of 1853.[10]

This is the largest reported outbreak to date of laboratory-confirmed cases of waterborne giardiasis (703 cases). Previous investigations of waterborne giardiasis outbreaks have described a larger number of possible cases and community-wide attack rates of clinical illness ranging from 3.8 per cent to 10.6 per cent.[11-15] It is likely that those who obtained a laboratory diagnosis of their infection in Pittsfield represented only a portion of the infected population: the survey indicates 1.9 additional symptomatic suspect cases for every one laboratory-confirmed case. Additional persons were likely to have had asymptomatic infection. Estimates of numbers of infected persons in outbreaks depend upon the sensitivity and specificity of the case definition and on the accuracy and validity of telephone surveys.

Community health officials were encouraged by the high degree of reported compliance with their recommendations to avoid drinking unboiled municipal water. However, few people actually boiled tap water; most people were using alternate sources of drinking water although all were aware of the boil-water order.

It is ironic that this outbreak occurred as a result of the attempt to install a filtration system that would probably have prevented the outbreak. Many small communities lack filtered water supplies; they are costly and require technical expertise to maintain. In Massachusetts, among 124 surface water supplies, 76 had either no filtration system or partial filtration systems in 1985 (personal communication, John Higgins, Massachusetts Department of Environmental Quality Engineering). Giardiasis outbreaks may become even more frequent in the future, as increasing population pressure on watershed areas increase the risk of *Giardia* contamination for many communities that depend on unfiltered surface water supplies.

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REFERENCES

1. Craun GF: Waterborne giardiasis in the United States 1965–1984. Lancet 1986; 2:513–514.
2. Young KH, Bullock SL, Melvin DM, Spruill CL: Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. J Clin Microbiol 1979; 10:852–853.
3. Breslow NE, Day NE: *In:* Statistical Methods in Cancer Research, Volume I—The Analysis of Case-Control Studies. Lyon: International Agency for Research on Cancer, 1980; 248–281.
4. Kish L: *In:* Survey Sampling. New York: John Wiley and Sons, 1965; 113–144.
5. Mendenhall W, Ott L, Scheaffer R: *In:* Elementary Survey Sampling. Belmont, California: Duxbury Pres, 1979; 137–139.
6. Jakubowski W, Chang SL, Erickson TH, *et al:* Large volume sampling of water supplies for micro-organisms. J Am Water Works Assoc 1978; 70:702–706.
7. Sauch JF: Use of immunofluorescence and phase-contrast microscopy for detection and identification of *Giardia* cysts in water samples. Appl Environ Microbiol 1985; 50:1434–1438.
8. Belosevic M, Faubert GM, MacLean JD, Law C, Croll NA: *Giardia lamblia* infections in Mongolian gerbils: an animal model. J Infect Dis 1983; 147:222–226.
9. Jarroll EL, Bingham AK, Meyer EA: Effect of chlorine on *Giardia lamblia* cyst viability. Appl Environ Microbiol 1981; 41:483–487.
10. Snow J: Snow on Cholera. London: Oxford University Press, 1936.
11. Veazie L, Brownlee I, Sears JH: An outbreak of gastroenteritis associated with *Giardia lamblia*. *In:* Waterborne Transmission of Giardiasis. Cincinnati: US Environmental Protection Agency, 1979; 174–191.
12. Shaw PK, *et al:* A community-wide outbreak of giardiasis with documented transmission by municipal water. Ann Intern Med 1977; 87:426–432.
13. Dykes AC, *et al:* Municipal waterborne giardiasis: an epidemiologic investigation. Ann Intern Med 1980; 92:165–170.
14. Lopez CE, *et al:* Waterborne giardiasis: a community-wide outbreak of disease and high rate of asymptomatic infection. Am J Epidemiol 1980; 112:495–507.
15. Navin TR, *et al:* Case-control study of waterborne giardiasis in Reno, Nevada. Am J Epidemiol 1985; 122:269–275.

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A Waterborne Outbreak of Cryptosporidiosis in Normal Hosts

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In July 1984, an outbreak of gastroenteritis occurred in a suburban community in Texas. A random telephone survey of 100 of 1791 households in the community identified an attack rate of 34%. The outbreak was traced to contamination of the community water supply, an artesian well. Fecal coliforms were identified in untreated drinking water from the well during July. Stool examinations and serologic tests identified *Cryptosporidium* as the etiologic agent. *Cryptosporidium* should be added to the list of waterborne organisms capable of causing outbreaks of gastroenteritis.

CRYPTOSPORIDIUM has been recognized recently as an important cause of diarrhea in immunocompromised (1) and immunocompetent persons (2, 3). Most cryptosporidium infections are reported to occur in animal handlers and patients with the acquired immunodeficiency syndrome. In patients with the syndrome, this parasite causes a prolonged and debilitating diarrheal illness (1); in immunocompetent adults, it produces a self-limited diarrheal illness lasting an average of 5 days (2, 3). Two recent reports described outbreaks of cryptosporidiosis in children attending day-care centers (4, 5), and in surveys of patients with diarrhea, prevalence rates as high as 4% have been reported in Australia and Costa Rica (6, 7). Infection with this organism may be more common than previously suspected.

In July 1984, a waterborne outbreak of diarrhea occurred among residents of Braun Station, a suburb of San Antonio, Texas. The outbreak was caused by *Cryptosporidium*.

Methods

Stool specimens from Braun Station residents and outpatients treated at the Wilford Hall Medical Center, Lackland Air Force Base, in July 1984 were examined for ova and parasites at the parasitology laboratory of the medical center or at the Bureau of Laboratories, Texas Department of Health. Fresh and formalin-preserved stool specimens were concentrated by a modification of Ritchie's formalin-ether concentration method (8) using ethyl acetate instead of ether. Concentrated specimens were examined by light microscopy with iodine stain. To identify *Cryptosporidium* oocysts, air-dried, heat-fixed smears of fecal concentrates were stained by Truant's fluorescent procedure (9) and a modified Kinyoun's acid-fast stain (10). Fecal films of specimens preserved in polyvinyl alcohol were stained by Wheatley's trichrome method (11). Stool specimens were examined for bacterial pathogens including *Shigella*, *Salmonella*, *Campylobacter*, and *Yersinia* by routine microbiologic techniques. Isolation of viruses was not attempted. Total and fecal coliform counts in water samples were made by standard procedures at the San Antonio Metropolitan Health District Laboratory. Serum specimens from Braun Station residents were tested for antibody to Norwalk virus by radioimmunoassay (12), for antibody to *Cryptosporidium* by an indirect immunofluorescent technique (13).

A telephone survey of community households was done during 25 through 27 July 1984. Pages in the residential telephone directory for San Antonio were selected from a table of random numbers. The first number listed on each page with a prefix corresponding to northwest San Antonio was selected.

Persons contacted by telephone were asked to verify the street address, provide the number and names of household members, their ages and sex, occurrence of any illnesses in May and July, and week of onset of symptoms. The first person in each household who was ill with diarrhea or vomiting, or if no one was ill, the person answering the telephone, was asked additional questions about symptoms; duration of illness; dates of overnight trips; pets; contact with farm animals; children enrolled in day-care centers; recent swimming in pools, lakes, or rivers; place of employment; recent consumption of raw seafood or raw milk; source of drinking water; and types of fluids ingested daily. A total of 100 Braun Station households and 50 households outside Braun Station were surveyed. In addition, all Braun Station residents with stool specimens positive for *Cryptosporidium* were interviewed with this questionnaire. Statistical analyses were done with the chi-square test and Mantel-Haenszel chi-square test (14).

Results

Braun Station is a suburban community of approximately 5900 persons located 20 miles northwest of San Antonio, Texas. Potable water was supplied to all households from the same artesian well. Water was not filtered before use but it was chlorinated with gas shortly before distribution. We surveyed 100 of 1791 households in Braun Station for a total of 346 persons. Our investigation showed two distinct outbreaks of gastroenteritis in the community between 1 May and 30 July 1984.

The first outbreak in May was identified retrospectively. We found that 251 persons (72%) in 85 households had an acute gastrointestinal illness. Only 2.5% of the 120 persons living in the 50 households outside Braun Station had diarrhea or vomiting in May ($p < 0.00001$, chi-square test). The dates of onset of symptoms for the first ill persons in 74 households are shown in Figure 1. Dates of onset were unknown for 11 persons. Only six serum specimens had been collected in May at the time of

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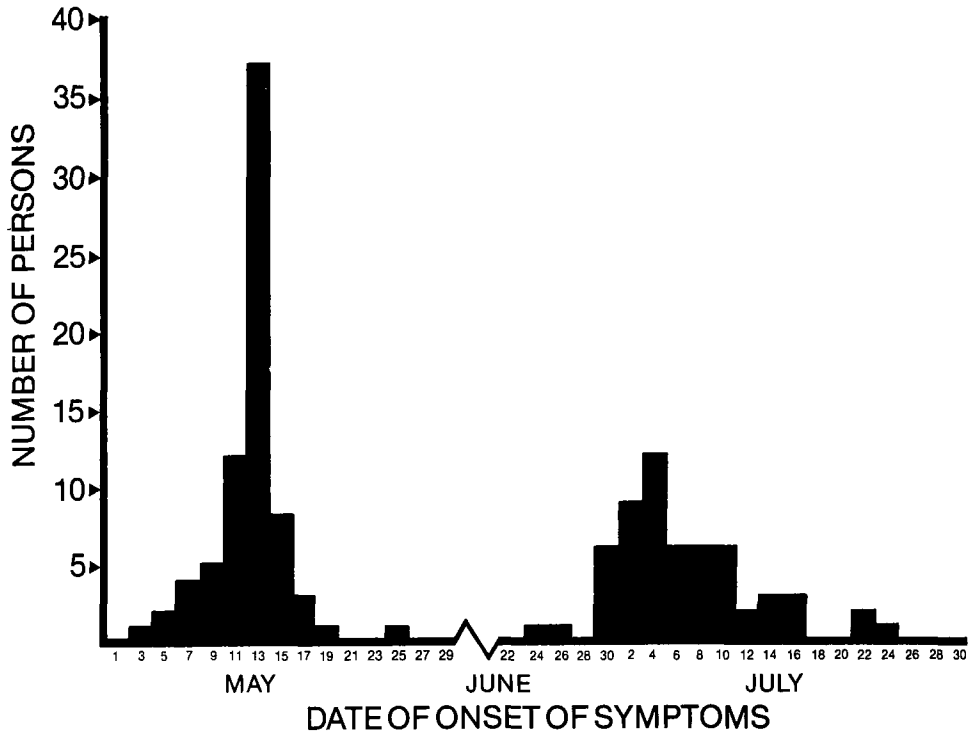

Figure 1. Dates of onset of symptoms in the first ill family members in 100 surveyed households in Braun Station, Texas, May and July 1984.

the outbreak, but these as well as convalescent sera were tested for Norwalk virus antibodies. Four specimens had fourfold rises in titer to Norwalk virus and the remaining two had seroconverted from undetectable to low titers. Because no sera were available from well persons, further serologic testing was not done on them. Symptoms had been similar to those in other outbreaks of Norwalk gastroenteritis.

The second outbreak was investigated prospectively. In July 1984, 117 persons (34%) in 60 households had a gastrointestinal illness. Only 2.5% of persons residing outside Braun Station were ill in July ($p = 0.00001$, chi-square test). No geographic clustering of households with ill persons was evident, and the ill persons ranged in age from 1 to 72 years with a mean of 32 years. Symptoms began during the week of 1 July in 53% and during the week of 8 July in 20%. Dates of onset of symptoms for the first ill persons are presented in Figure 1.

The symptoms of persons in the 60 Braun Station households are presented in Table 1. Most had diarrhea and abdominal cramps that lasted from 1 to 25 days, with a mean of 6 days. No one was immunocompromised.

No association was evident between pet ownership, exposure to farm animals, or swimming in the community pool and occurrence of diarrhea in July. There was, however, an inverse association between the occurrence of diarrheal illness in July and taking a vacation between 1 and 11 July. Twenty-one percent of persons who took a vacation in this period developed a diarrheal illness compared with 72% of those who remained in Braun Station ($p = 0.00006$, chi-square test). Analysis for each day during this interval indicated that absence from the community on July 2 was most protective ($p = 0.01$, chi-square test). A linear relation was seen between the proportion of total daily fluid intake of tap water or tap water equivalents (reconstituted frozen juices, instant iced tea, and powdered drinks) and the occurrence of diarrhea in July: the greater the proportion of daily fluid intake of tapwater, the higher the attack rates of diarrhea ($p = 0.002$, Mantel-Haenszel chi-square test for trend) (data not presented).

Cryptosporidium oocysts were identified in the stools of 47 of 79 residents who became ill in July. Forty of these forty-seven persons had diarrhea, 12 had vomiting, 12 had fever, and 2 were asymptomatic. One was hospitalized. Only 12 of 194 stool specimens submitted by outpatients who did not reside in Braun Station but who were treated for gastroenteritis at the Wilford Hall Medical Center contained *Cryptosporidium* oocysts. Four of these persons had had person-to-person contact or drank tap water in Braun Station during July. Twenty-three stool specimens that contained *Cryptosporidium* oocysts were cultured for bacterial pathogens. *Salmonella enteriditis* was identified in 1 and *Giardia lamblia* cysts were identified in 5. Only 3 of the 32 Braun Station families with cryptosporidiosis had children in a day-care center.

Forty-three persons with a stool positive for *Cryptosporidium* submitted at least one additional stool. Nineteen passed oocysts for at least 5 days and 9 passed oocysts for at least 20 days. One person continued to pass oocysts 30 days after his first positive specimen was submitted. Paired serum specimens were obtained from 12 residents with positive stool. None showed detectable titers to *Cryptosporidium* in acute specimens, but 9 developed titers of 1:40 or greater in convalescent specimens.

Untreated water samples from wells in this part of Texas are not usually tested. Pretreatment water samples, however, were examined beginning in mid-June because

Table 1. Symptoms and Duration of Illness in a Cryptosporidiosis Outbreak in July 1984 at Braun Station, Texas

	Ill Persons Reporting Symptoms
Symptoms, %	
Diarrhea	100
Abdominal cramps	76
Nausea	45
Vomiting	19
Fever	14
Headache	29
Muscle aches	13
Duration of illness, *d*	
Mean	6
Range	1-25

of community complaints. Although chlorinated water samples collected from residences remained negative for coliforms, nine samples of untreated well water collected between 2 and 11 July contained fecal coliforms. Samples collected on 5 and 6 July contained 2600 and 1300 counts/100 mL, respectively. An order to boil water before drinking was issued on 11 July and remained in effect until the community was provided with an alternate water supply later that month. Subsequent water tests showed that dye introduced into the community's sewage system appeared in the well water. Attempts to identify the exact site of surface water or sewage contamination were unsuccessful.

Discussion

Two outbreaks of gastroenteritis occurred in a single suburban community in Texas in the summer of 1984, and evidence shows that both were caused by contamination (probably intermittent) of the community's water supply. In both outbreaks, the incidence of diarrheal illness in Braun Station residents was at least 12 times as high as that in neighboring communities with a different water supply.

In the July outbreak, the wide age range of ill persons, the lack of geographic clustering, the strong protection afforded to persons on vacation during the first week in July, and analysis of tap water consumption indicate that the outbreak was transmitted through the community water supply. Coliform tests of unchlorinated water confirm that the water supply was contaminated between 2 and 11 July. Limited laboratory data indicate that the major pathogen in the first outbreak was Norwalk virus, whereas the major pathogen in the second outbreak was *Cryptosporidium*, indicating intermittent contamination of the water supply.

The typical clinical manifestations produced by *Cryptosporidium* in immunocompetent adults are not well defined. Preliminary evidence from outbreaks in children at day-care centers shows that it can produce a short-lived diarrheal illness accompanied by very little vomiting or fever (4, 5). The symptoms of children in the July outbreak were generally milder than those of young adults. Diarrhea was the most prominent symptom, as well as the longest lasting, in adults. Significant volume depletion occurred in two persons, one of whom was hospitalized for fluid therapy. Because of the prolonged nature of the diarrhea, weight loss occurred in 41% of the ill adults. A proper estimate of the incidence of asymptomatic cryptosporidiosis could not be calculated, but asymptomatic cryptosporidiosis clearly occurred in some family members of households with proven cases. In some persons the fecal shedding of the organism was prolonged. Sixty-nine percent of persons tested had stool specimens positive for *Cryptosporidium* after their clinical symptoms had resolved.

Our report describes the largest common-source outbreak of cryptosporidiosis to date. The epidemiologic data show that the source was fecally contaminated water. The results also suggest that *Cryptosporidium*, like *Giardia*, may be resistant to the usual levels of chlorination of potable water supplies. *Cryptosporidium* should be added to the list of waterborne microorganisms capable of causing gastroenteritis.

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References

1. Soave R, Danner RL, Honig CL, et al. Cryptosporidiosis in homosexual men. *Ann Intern Med.* 1984;**100**:504-11.
2. Current WL, Reese NC, Ernst JV, Bailey WS, Heyman MB, Weinstein WM. Human cryptosporidiosis in immunocompetent and immunodeficient persons: studies of an outbreak and experimental transmission. *N Engl J Med.* 1983;**308**:1252-7.
3. Wolfson JS, Hopkins CC, Weber DJ, Richter JM, Waldron MA, McCarthy DM. An association between cryptosporidium and giardia in stools [Letter]. *N Engl J Med.* 1984;**310**:788.
4. Alpert G, Bell LM, Kirkpatrick CE, et al. Cryptosporidiosis in a day-care center [Letter]. *N Engl J Med.* 1984;**311**:860-1.
5. Centers for Disease Control. Cryptosporidiosis among children attending day-care centers—Georgia, Pennsylvania, Michigan, California, New Mexico. *MMWR.* 1984;**33**:599-601.
6. Tzipori S, Smith M, Birch C, Barnes G, Bishop R. Cryptosporidiosis in hospital patients with gastroenteritis. *Am J Trop Med Hyg.* 1983;**32**:931-4.
7. Mata L, Bolanos H, Pizarro D, Vives M. Cryptosporidiosis in children from some highland Costa Rican rural and urban areas. *Am J Trop Med Hyg.* 1984;**33**:24-9.
8. Ritchie LS. An ether sedimentation technique for routine stool examinations. *Bull US Army Med Dept.* 1948;**8**:326.
9. Truant JP, Brett WA, Thomas W. Fluorescence microscopy of tubercle bacilli stained with auramine and rhodamine. *Henry Ford Hosp Med Bull.* 1962;**10**:287.
10. Paik G, Sugg MT. Reagents, stains, and miscellaneous test procedures. In: Lennette, EH, Spaulding EH, Truant JP, eds. *Manual of Clinical Microbiology.* 2nd ed. Washington D.C.: American Society for Microbiology; 1974:930-50.
11. Wheatley WB. A rapid staining procedure for intestinal amoebae and flagellates. *Am J Clin Pathol.* 1951;**21**:990-2.
12. Greenberg HB, Wyatt RG, Valdesuso J, et al. Solid-phase microtiter radioimmunoassay for detection of the Norwalk strain of acute nonbacterial, epidemic gastroenteritis virus and its antibodies. *J Med Virol.* 1978;**2**:97-108.
13. Campbell PN, Current WL. Demonstration of serum antibodies to *Cryptosporidium* sp. in normal and immunodeficient humans with confirmed infections. *J Clin Microbiol.* 1983;**18**:165-9.
14. Schlesselman JJ. *Case-Control Studies.* New York: Oxford University Press; 1982.

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LARGE COMMUNITY OUTBREAK OF CRYPTOSPORIDIOSIS DUE TO CONTAMINATION OF A FILTERED PUBLIC WATER SUPPLY

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Abstract Between January 12 and February 7, 1987, an outbreak of gastroenteritis affected an estimated 13,000 people in a county of 64,900 residents in western Georgia. Cryptosporidium oocysts were identified in the stools of 58 of 147 patients with gastroenteritis (39 percent) tested during the outbreak. Studies for bacterial, viral, and other parasitic pathogens failed to implicate any other agent. In a random telephone survey, 299 of 489 household members exposed to the public water supply (61 percent) reported gastrointestinal illness, as compared with 64 of 322 (20 percent) who were not exposed (relative risk, 3.1; 95 percent confidence interval, 2.4 to 3.9). The prevalence of IgG to cryptosporidium was significantly higher among exposed respondents to the survey who had become ill than among nonresident controls.

Cryptosporidium oocysts were identified in samples of treated public water with use of a monoclonal-antibody test. Although the sand-filtered and chlorinated water system met all regulatory-agency quality standards, suboptimal flocculation and filtration probably allowed the parasite to pass into the drinking-water supply. Low-level cryptosporidium infection in cattle in the watershed and a sewage overflow were considered as possible contributors to the contamination of the surface-water supply.

We conclude that current standards for the treatment of public water supplies may not prevent the contamination of drinking water by cryptosporidium, with consequent outbreaks of cryptosporidiosis. (N Engl J Med 1989; 320:1372-6.)

HUMAN cryptosporidiosis was first described in 1976 in a three-year-old child with no obvious immunodeficiency.[1] It was subsequently recognized as a cause of severe diarrhea in patients with the acquired immunodeficiency syndrome and found to be a common cause of sporadic and epidemic gastroenteritis in immunocompetent persons.[2-13] Transmission from calves to humans, from person to person, and by a common source have all been described.[4,8,9,12] Outbreaks have occurred in day-care centers,[10,11] among international travelers,[3,13] and in a community supplied with unfiltered drinking water from a contaminated municipal well.[9]

We report the contamination of a filtered public water supply by cryptosporidium that occurred even though the treated water met federal and state standards for drinking water and that resulted in the largest outbreak of cryptosporidiosis described to date.

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BACKGROUND

In mid-January 1987 a college physician informed health authorities of a dramatic increase in gastroenteritis among students at West Georgia College in Carrollton, Georgia. Carrollton is the principal city in Carroll County (population, 64,900), located in western Georgia.

An initial investigation by state and federal epidemiologists indicated that the outbreak involved the entire community. The laboratory director at Carrollton's hospital reported that four stool specimens obtained from patients with acute gastroenteritis were positive for cryptosporidium. The first positive report had been on January 12.

An initial case–control study of patients presenting with gastroenteritis to the hospital emergency room and a comparison of gastroenteritis rates at nursing

homes connected to the public water system with rates at homes independent of the system implicated the water supply as a possible source of the outbreak. An advisory to boil water was issued on January 30.

METHODS

Surveillance and Laboratory Studies

To define the course of the epidemic, we reviewed emergency-room logs at the Carrollton hospital and recorded the number of visits of patients who presented each week with acute gastrointestinal illness from December 1986 through March 1987.

From January 20 through March 10, stool specimens for ova and parasite examination were obtained from 147 patients with gastroenteritis who presented to outpatient facilities. Seventy-six of these patients completed questionnaires on their symptoms at the time of the stool collection. Two aliquots of each stool were preserved separately in 10 percent formalin and in polyvinyl alcohol. The formalin-preserved stool was examined unstained, after iodine staining, and (after concentration by formalin–ethyl acetate sedimentation) after modified acid-fast and auramine–rhodamine staining.[14] Stool preserved with polyvinyl alcohol was stained with trichrome and examined by light microscopy. All stool samples were examined at the Parasitic Disease Laboratory of the Centers for Disease Control (CDC).

To evaluate the role of bacterial or viral pathogens, we reviewed the results of all bacterial stool cultures performed at the hospital laboratory during January, and we collected stool samples from five acutely ill college students for examination by immune electron microscopy for viral particles. In addition, paired serum samples obtained six weeks apart — during the acute phase of the illness and during convalescence — from 10 college students were tested for seroconversion to rotavirus by IgG and IgA enzyme-linked immunosorbent assay (ELISA). Seven of these paired serum samples were also analyzed for the presence of antibody to Norwalk virus with use of the avidin–biotin test. Viral studies were done at the Gastroenteritis Laboratory of the CDC.

Telephone Survey

From January 31 to February 2, the Carroll County Health Department surveyed by telephone 304 households systematically sampled from the county telephone directory. Carrollton households were oversampled to ensure an adequate sample of persons who had been exposed to the public water supply.

Adult respondents (over 18 years of age) were asked about the source of their home water, their age, their sex, the place of employment of all household members, and whether any household member had been ill with abdominal pain or diarrhea since January 1. They were also asked about their consumption of specific foods, their use of restaurants, the amount of tap water they consumed, and their exposure to children in day-care centers and to farm animals. Data were obtained on all 304 respondents and 507 additional household members. County engineers determined the water supply for given work sites and schools. People whose home, school, or work site was supplied with public water were considered to have been exposed to the public water supply.

From March 7 through 12, we were able to obtain serum samples from 86 of the 159 telephone-survey respondents who were living in Carrollton and had been exposed to the public water supply. These samples and randomly selected, banked control serum samples from 20 CDC employees who had not traveled outside the United States were tested for the presence of IgG and IgM to cryptosporidium by ELISA[15] at the Uniformed Services University of the Health Sciences in Bethesda, Maryland.

Investigation of the Water System

During the outbreak, federal and local engineers evaluated the water-treatment plant in accordance with published procedures.[16] Samples of raw and treated water from various points in the water system were examined for cryptosporidium and giardia by passing 379 to 3785 liters (100 to 1000 gal) of water through a 1-μm polypropylene filter. The eluted sediment was examined directly for giardia, and a fluorescein-tagged monoclonal antibody (Meridian Diagnostics) was used to detect cryptosporidium in the sediment at the Parasitic Disease Laboratory of the CDC, according to methods described by Jakubauski,[17] and at the Department of Microbiology and Immunology of the University of Arizona, according to methods described elsewhere.[18,19]

To evaluate the means of contamination of the water supply, we reviewed data on sewage and water maintenance and on rainfall, and examined fresh stool samples for cryptosporidium from 67 of the 226 cattle (including 3 calves) pastured in the watershed.

RESULTS

Surveillance and Laboratory Studies

The number of visits of patients with gastroenteritis to the hospital emergency room increased in the second week of January, peaked from the third week of January through the first week of February, and then declined to preoutbreak levels by the last week of February (Fig. 1).

Cryptosporidium was identified in the stools of 58 of the 147 patients with gastroenteritis (39 percent) tested during the outbreak. Giardia was detected in stool from only one subject, a two-year-old child who also had cryptosporidiosis.

Of the 70 stool samples cultured for bacteria at the hospital laboratory during January, only 2 were positive: 1 for shigella and the other for campylobacter. The results of all viral studies in the 10 college students who became ill were negative.

Information on signs and symptoms was obtained from 30 outpatients with cryptosporidium-positive stools. These patients ranged in age from less than 1 year to 46 years (mean age, 20). Diarrhea (defined as three or more loose bowel movements per day) was reported by 87 percent, stomach pain by 80 percent, nausea by 67 percent, vomiting by 33 percent, fever by 30 percent, and muscle aches by 20 percent.

Telephone Survey

Of the 811 household members surveyed by telephone, 363 had been ill with diarrhea or abdominal pain since January 1. After adjustment for the oversampling of the city of Carrollton, where the attack rate was 54 percent, the overall estimated attack rate for the county was 40 percent.

Of the 489 household members who were exposed to the public water supply, 299 (61 percent) had been ill, as compared with 64 of the 322 unexposed subjects (20 percent; relative risk, 3.1; 95 percent confidence interval, 2.4 to 3.9; Taylor series approximation).[20] When we used logistic regression models to control for the exposure to the public water supply, we found no strong or significant association between illness and any other risk factor studied (i.e., exposure to day-care centers, farm animals, or particular restaurants; household size; or consumption of chicken or beef).

Among the 489 exposed household members, the attack rate was 67 percent (170 of 254) in female and 55 percent (128 of 232) in male household members (the sex of 3 subjects was not recorded) (relative risk, 1.2; 95 percent confidence interval, 1.0 to 1.4).

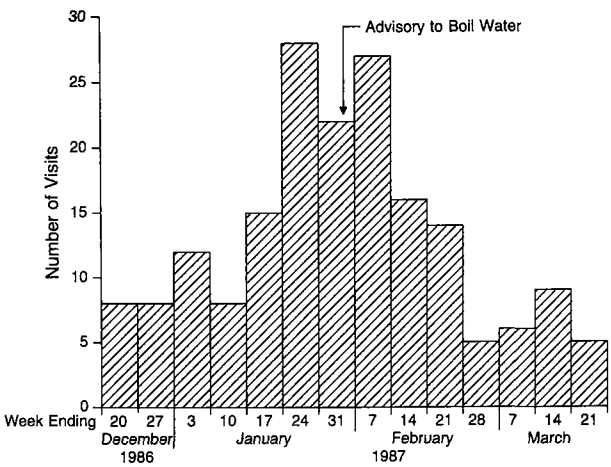

Figure 1. Numbers of Visits of Patients with Gastroenteritis to the Hospital Emergency Room in Carroll County, Georgia, According to the Week of the Visit.

Among the exposed respondents, the higher attack rate in female members remained significant when we controlled for age and water consumption. The attack rates according to age group among all 489 exposed household members ranged from 52 to 72 percent and were highest among those 20 to 29 years of age, but these differences were not statistically significant (P>0.05 by the chi-square test for independence).

The telephone survey indicated that approximately 50 percent of Carroll County residents, or 32,450 people, were exposed to the public water supply at home or at work. The risk of illness attributable to this exposure was 41 percent (61 percent of exposed members who became ill minus 20 percent of unexposed members who became ill). We therefore estimate that approximately 13,000 persons had an illness attributable to public-water exposure during the outbreak.

For the 86 exposed respondents to the telephone survey who provided serum samples, the prevalence of detectable IgG to cryptosporidium was higher among the 68 respondents who had become ill than among the 18 who had not become ill (76 percent vs. 56 percent; P = 0.08 by the chi-square test), and it was significantly higher among respondents who had become ill than among the 20 CDC employees (76 percent vs. 35 percent; P = 0.001 by the chi-square test). The seroprevalence of cryptosporidium-specific IgM with IgG was 12 percent in respondents who became ill, 6 percent in those who had not become ill, and 5 percent in the CDC controls (P>0.50, by Fisher's exact test for each comparison).

Investigation of the Water System

Roughly 7900 of the 19,000 households in the county receive water from the Carrollton water system. The remainder have wells or are connected to separate municipal water systems. The Carrollton water system draws water from a small river that runs through surrounding pasture land. The water is treated by the addition of alum, lime, and chlorine, rapid mixing, mechanical flocculation (a process to promote the aggregation of particulates), sedimentation, and rapid sand filtration.

Samples obtained from the municipal water system on January 5, 22, and 23 were tested for coliform bacteria by standard methods[21] and were all negative. All routine measurements of treated water were within the limits set by the Environmental Protection Agency and the State of Georgia for turbidity, coliform bacteria, and residual free chlorine. Nevertheless, on January 28, samples of treated water contained particles as large as 100 μm (cryptosporidium oocysts are 4 to 6 μm in size).

Cryptosporidium oocysts were ultimately identified in samples of treated water taken from the water-treatment plant on January 28, February 4, and February 5 and from four dead-end water mains, including one at the college, between February 4 and 6. Two samples of raw water obtained from streams that drain into the river above the treatment plant were positive for cryptosporidium, but five samples taken from the river itself, upstream from the treatment plant, were all negative. Three samples of treated water taken as controls from a nearby town with a separate water source were also negative.

No outbreaks of diarrheal illness were reported among cattle pastured in the county. Cryptosporidium was found in low numbers in stool samples obtained from 3 of the 56 cattle tested from pastures along the river upstream from the water plant (2 of the positive stool samples were from calves), but it was absent in the stool samples from 11 cattle tested from pastures along the streams in which cryptosporidium was found. There were no major rainfalls in early January, but more than 25 cm (10 in) of rain and snow fell from January 15 to 22.

Although no serious breaks in the water or sewage systems were reported during January, a sewage overflow caused by a blocked major sewer line was discovered in mid-February in a wooded area above the water-treatment plant. When dye was released at the overflow point, it reached the river and the water-treatment plant in approximately six hours. We could not determine when the sewage pipe had begun to overflow. An analysis of the surface-water supply for coliform bacteria after the sewage spill had been cleaned up found no indication of ongoing discharge of raw sewage.

We attributed the passage of particulates through the treatment plant to three factors. First, mechanical agitators that were scheduled for replacement had been removed from the flocculation basins in December in anticipation of the arrival of new agitators. This reduced the efficiency of the flocculation step and impaired particulate removal. Second, the efficiency of filtration was impaired by the equipment and procedures used to control the flow of water through the filters and to monitor turbidity. Finally, the filters were sometimes restarted without first being back-

washed. This could have discharged dirt, flocculent particles, and microorganisms from the filter beds into the treated water. The Environmental Protection Agency has set a turbidity limit for water entering the distribution system of 1 nephelometric turbidity unit (NTU) or less, measured at least once daily. The Carrollton water system met this regulation; however, turbidity measurements of water obtained from individual filters on February 3 showed that three properly backwashed and restarted filters were producing water with a turbidity of 0.07 to 0.18 NTU, whereas three filters restarted without being backwashed produced water with a turbidity ranging from 0.2 to 3.2 NTU in the first three hours after they were restarted. During the first week of January the number of filters restarted without undergoing backwashing increased to 38 from a usual weekly average of 22, because water use increased after the holidays.

The water-treatment process was improved in February so that turbidity of continuously monitored treated water was 0.2 NTU or less — a value consistent with the removal of particles larger than approximately 1 μm. A sample of treated water taken on February 11 showed no oocysts. The consistently low turbidity readings resulted in the lifting of the advisory to boil water on March 2. Clinical and laboratory surveillance through the end of May showed no recurrence of cryptosporidiosis in Carroll County.

Discussion

To our knowledge, this is the first reported contamination of a filtered water system by cryptosporidium. The sudden onset of widespread gastroenteritis affecting persons of all ages is typical of a waterborne outbreak. There was a strong association of illness with exposure to the public water supply among the county residents surveyed by telephone and a high prevalence of cryptosporidium-specific IgG in exposed respondents to the survey who had been ill. Cryptosporidium oocysts were identified in the stools of patients during the outbreak as well as in the treated public water. Neither laboratory examination of patients nor evaluation of the water system yielded any evidence that other enteric pathogens contributed to the outbreak.

Although there was adequate chlorine in the water to inactivate most known bacteria and viruses,[22] cryptosporidium oocysts are highly resistant to chlorine.[23] The efficacy of boiling water to inactivate cryptosporidium is uncertain, but temperatures above 100°C kill all other protozoa that have been tested, and inactivation of cryptosporidium oocysts by exposure to a moist heat (55°C) for 20 minutes has been demonstrated. Although we have no information on the residents' compliance with the advisory to boil water, it appears, in conjunction with the improvements in water-treatment procedures, to have controlled the outbreak.

Exposed persons were defined as county residents with access to the public water supply at home, school, or work. Since Carrollton is the commercial center of the county, it is likely that a number of persons who were considered to be unexposed in our study were actually exposed to the public water supply during recreational or commercial visits to Carrollton. This may explain the illness rate of 20 percent among those considered unexposed in the study.

The higher attack rate among female residents in the survey remains unexplained. Surveys in settings with no outbreak have found a higher rate of self-reported diarrheal illness in women than in men (Ho MS: unpublished data), but our data do not allow us to determine whether the sex-based difference we observed was due to a difference in reporting behavior or susceptibility to symptomatic infection.

Cryptosporidiosis is an illness of variable severity. Many patients reported mild, nonspecific symptoms during the outbreak in Carroll County. Of those with cryptosporidiosis confirmed by stool culture, 13 percent did not have diarrhea, as defined by three or more loose stools per day; therefore, the diagnosis of cryptosporidiosis should not be excluded on the basis of the absence of diarrhea.

The prevalence of cryptosporidium-specific IgG was higher among exposed survey respondents who had not been ill than among CDC employees (56 percent vs. 35 percent) and among a group of Peace Corps volunteers tested before placement overseas (of 75 subjects, 24, or 32 percent, had IgG to cryptosporidium).[24] Although not statistically significant, these differences suggest that before the outbreak, the background exposure to cryptosporidium among residents of Carroll County was higher than the typical background exposure among U.S. residents, or that many residents had asymptomatic infection during the outbreak. Since the stool samples examined for the presence of ova and parasites from the Carrollton area were not routinely examined for cryptosporidium before November 1986, previous cases of cryptosporidiosis were not likely to have been detected.

Infected cattle in the watershed could cause long-term low-level contamination of the surface water; however, we could not conclude that the outbreak was caused directly by cryptosporidiosis in cattle, since the level of infection in cattle was low and the distribution of cattle that tested positive did not match the distribution of positive water samples.

Regardless of the source of contamination of the untreated surface-water supply, we believe that the changes in water treatment around the holiday season allowed oocysts to pass into the treated water in sufficient numbers to cause widespread illness. In addition, the sewage spill above the treatment plant and the increase in rainfall just after the onset of the outbreak may have increased the load of cryptosporidium reaching the plant, but this remains speculative.

Contamination of rivers and streams by cryptosporidium has been reported in several states.[25] The results of our investigation demonstrate that cryptosporidium can contaminate filtered public water systems

— even when the water quality is within regulatory limits for coliform bacteria, chlorine, and turbidity — causing large epidemics of gastroenteritis in otherwise healthy persons. Further study is needed to assess the prevalence and the effect on public health of cryptosporidium in surface-water supplies and to evaluate methods of preventing future contamination of public drinking water by this hardy parasite.

We are indebted to Mr. Olan Ledford for assistance in coordinating and organizing work involving the Carroll County Health Department; to Dr. Mary Miles for bringing the outbreak to our attention and for assistance in collecting specimens and epidemiologic data; to Dr. Charles Gerba and the laboratory staff at the University of Arizona for the application of the most recent water-sampling technology; to Dr. Henry Mathews, Dr. Govinda Visvesvara, and Ms. Jennifer Dickerson, CDC, for examining the stool and water specimens; to Dr. Roger Glass and Dr. Mei-Shang Ho, CDC, for viral studies; to Dr. Bernard Nahlen, CDC, for assisting the investigation; to Mr. Lewis Mason and Mr. Jim Baxley, Carrollton City and County Water Utilities, for assistance in evaluating the water system; to Mr. Ted Mikalsen, Georgia Department of Natural Resources, for testing streams in the watershed for coliform bacteria; to Ms. Cheri Toni for help in case finding and surveillance; to Ms. Shirley Johnson for administrative assistance; to Ms. Robbie Green for notifying us of the sewage overflow; to Dr. Keith Sikes, Dr. Jeffrey Sacks, and Mr. David Smith, Georgia Department of Human Resources, for assisting the investigation; to the staffs of the college, the hospital, and the Carroll County Health Department for their assistance; and to Ms. Voughn Trader for assistance in the preparation of the manuscript.

References

1. Nime FA, Burek JD, Page DL, Holscher MA, Yardley JH. Acute enterocolitis in a human being infected with the protozoan Cryptosporidium. Gastroenterology 1976; 70:592-8.
2. Holley HP Jr, Dover C. *Cryptosporidium:* a common cause of parasitic diarrhea in otherwise healthy individuals. J Infect Dis 1986; 153:365-8.
3. Jokipii L, Jokipii AMM. Timing of symptoms and oocyst excretion in human cryptosporidiosis. N Engl J Med 1986; 315:1643-7.
4. Current WL, Reese NC, Ernst JV, Bailey WS, Heyman MB, Weinstein WM. Human cryptosporidiosis in immunocompetent and immunodeficient persons: studies of an outbreak and experimental transmission. N Engl J Med 1983; 308:1252-7.
5. Navin TR, Juranek DD. Cryptosporidiosis: clinical, epidemiologic, and parasitologic review. Rev Infect Dis 1984; 6:313-27.
6. Soave R, Armstrong D. *Cryptosporidium* and cryptosporidiosis. Rev Infect Dis 1986; 8:1012-23.
7. Fayer R, Ungar BL. Cryptosporidium spp. and cryptosporidiosis. Microbiol Rev 1986; 50:458-83.
8. Human cryptosporidiosis — Alabama. MMWR 1982; 31:252-4.
9. D'Antonio RG, Winn RE, Taylor JP, et al. A waterborne outbreak of cryptosporidiosis in normal hosts. Ann Intern Med 1985; 103:886-8.
10. Alpert G, Bell LM, Kirkpatrick CE, et al. Outbreak of cryptosporidiosis in a day-care center. Pediatrics 1986; 77:152-7.
11. Cryptosporidiosis among children attending day-care centers — Georgia, Pennsylvania, Michigan, California, New Mexico. MMWR 1984; 33:599-601.
12. Koch KL, Phillips DJ, Aber RC, Current WL. Cryptosporidiosis in hospital personnel: evidence for person-to-person transmission. Ann Intern Med 1985; 102:593-6.
13. Jokipii L, Pohjola S, Jokipii AM. Cryptosporidiosis associated with traveling and giardiasis. Gastroenterology 1985; 89:838-42.
14. Ma P, Soave R. Three-step stool examination for cryptosporidiosis in 10 homosexual men with protracted watery diarrhea. J Infect Dis 1983; 147:824-8.
15. Ungar BL, Soave R, Fayer R, Nash TE. Enzyme immunoassay detection of immunoglobulin M and G antibodies to *Cryptosporidium* in immunocompetent and immunocompromised persons. J Infect Dis 1986; 153:570-8.
16. Logsdon GS. Evaluating treatment plants for particulate contaminant removal. J Am Water Works Assoc 1987; 79(9):82-92.
17. Jakubauski W. Detection of *Giardia* cysts in drinking water: state of the art. In: Erlandsen SL, Mayer EA, eds. Giardia and giardiasis: biology, pathogenesis and epidemiology. New York: Plenum Press, 1984:263-85.
18. Rose JB. Detection of *Cryptosporidium* from wastewater and freshwater environments. Water Sci Technol 1986; 18:233.
19. Musial CE, Arrowood MJ, Sterling CR, Gerba CP. Detection of *Cryptosporidium* in water by using polypropylene cartridge filters. Appl Environ Microbiol 1987; 53:687-92.
20. Kleinbaum DG, Kupper LL, Morgenstern H. Epidemiologic research: principles and quantitative methods. Belmont, Calif.: Lifetime Learning Publications, 1982:296-9.
21. American Public Health Association, American Water Works Association, Water Pollution Control Federation, Georgia Department of Natural Resources. Standard methods for the examination of water and wastewater. 16th ed. New York: American Public Health Association, 1985.
22. Laubusch EJ. Chlorination and other disinfection processes. In: American Water Works Association. Water quality and treatment: a handbook of public water supplies. 3rd ed. New York: McGraw-Hill, 1971:201.
23. Campbell I, Tzipori AS, Hutchison G, Angus KW. Effect of disinfectants on survival of *Cryptosporidium* oocysts. Vet Rec 1982; 11:414-5.
24. Ungar BLP, Mulligan M, Nutman TB. Serologic evidence of *Cryptosporidium* infection in US volunteers before and during Peace Corps service in Africa. Arch Intern Med 1989; 149:894-7.
25. Rose JB. Occurrence and significance of *Cryptosporidium* in water. J Am Water Works Assoc 1988; 80(2):53-8.

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WATERBORNE OUTBREAK OF CRYPTOSPORIDIOSIS

SIR,—Despite the fact that *Cryptosporidium* spp have animal reservoir hosts and are transmitted by the faecal-oral route, *Cryptosporidium*, unlike *Giardia duodenalis*, has not yet been acknowledged widely as a significant cause of waterborne gastroenteritis. In 1985, D'Antonio et al[1] described the first waterborne outbreak, in Texas. Rush et al[2] reported *Cryptosporidium* spp oocysts in raw drinking water samples and postulated waterborne spread to account for a peak in cases of cryptosporidiosis in the Sheffield area in 1986. Hayes et al[3] described a waterborne outbreak in Georgia, USA, affecting 13 000 people exposed to a sand-filtered, chlorinated public water supply.

During April, 1988, several cases of cryptosporidiosis were reported to the community medicine specialist in Ayrshire. Extensive epidemiological investigations failed to implicate a common source of food or milk or consistent history of animal contact. Mapping of residences of the affected cases revealed that many of the households concerned received the same public potable water supply. 27 patients either resident in the area served by that supply or who consumed that water were diagnosed as *Cryptosporidium* spp positive (age range 4 months to 93 years), of whom 12 were admitted to hospital (11 required intravenous fluid replacement).

Following notification of the possible involvement of the water supply and the introduction of booster chlorination procedures, the water supply system was thoroughly examined. Twice during the outbreak *Escherichia coli*, coliforms, and faecal streptococci were isolated[4] from a service tank at the treatment works, but all other samples proved negative bacteriologically. *Cryptosporidium* spp oocysts were detected by Wright-Giemsa, modified Ziehl-Neelsen, saffranin/methylene-blue, phenol-auramine, and monoclonal antibody staining in the treated, chlorinated water supply system. Oocysts were present in sludge samples from the treatment works, in the final water leaving one of the service tanks, and in a break-pressure tank delivering chlorinated final water to the supply (range 0·13 to 1000 oocysts/l). Reinspection of the break-pressure tank revealed an old 50 cm fireclay pipe discharging into it; the pipe collecting run-off from the surrounding area. Water samples from the pipe and a nearby stream showed evidence of faecal contamination (244 *E coli*, 403 coliforms, 58 faecal streptococci per 100 ml, and 107 *E coli*, 152 coliforms, 34 faecal streptococci, per 100 ml, respectively). Samples from the stream and soil and grass adjacent to the fireclay pipe were positive for oocysts (0·13 oocyst/l and 32 oocysts/g, respectively). Oocysts were also detected in samples of the treated supply in the absence of faecal bacterial indicators. Following the isolation, drainage and disinfection of the contaminated storage tanks, extensive mains flushing, and emergency booster chlorination, no new cases of waterborne cryptosporidiosis have been reported, and bacteriological and parasitological analyses of subsequent water samples have proved negative.

Oocysts appear to have been introduced via the fireclay pipe into the break-pressure tank which contained final water for distribution rather than by failure of the water treatment process to retain them. There was evidence that cattle slurry had been sprayed on land in the vicinity of the fireclay pipe before the outbreak. *Cryptosporidium* spp oocysts can remain viable for at least 12 months at 4°C[5] and oocyst contamination of final waters in the absence of bacteriological contamination has been reported.[3] Attainable levels of free chlorine following booster chlorination procedures are not oocysticidal.[5] The waterborne route should be considered when clusters of cryptosporidiosis associated with potable water occur, even in the absence of bacterial or viral contamination.

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1. D'Antonio RG, Winn RE, Taylor JP, et al. A waterborne outbreak of cryptosporidiosis in normal hosts. *Ann Intern Med* 1985; **103**: 886–88.
2. Rush BA, Chapman PA, Ineson RW. *Cryptosporidium* and drinking water. *Lancet* 1987; ii: 632.
3. Hayes EB, Matte TD, O'Brien TR, et al. Contamination of a conventionally treated filtered public water supply by *Cryptosporidium* associated with a large community outbreak of cryptosporidiosis. *N Engl J Med* (in press).
4. Anon. The bacteriological examination of drinking water supplies. London: HM Stationery Office, 1983.
5. Smith AL, Smith HV, Girdwood RWA, Carrington E. The effect of free chlorine on the viability of *Cryptosporidium* spp oocysts. *WRc Publ* PRU 2023-M. Medmeacham, Bucks: Water Research Centre, 1988.

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What Happens When the Multiple Barrier Concept is Neglected

Waterborne disease: occurrence is on the upswing

Edwin C. Lippy

Three of the waterborne disease outbreaks in 1979 typified problems common to many water systems. Investigation of the outbreaks identified as causative factors deficiencies in water treatment practices and plant operation and a breakdown in surveillance activities designed to protect public health. Preliminary information indicates that a total of 45 outbreaks occurred during 1979, increasing the annual average of outbreaks to 36 for the four-year period from 1976 to 1979. This represents a 50 percent increase over the 1971–75 average of 24. The five-year averages have steadily increased from an annual average of ten during 1951–55. Before that period the trend was declining.

The disturbing trend toward more frequent occurrence of outbreaks of waterborne disease should serve as a warning to all who share in the responsibility for the delivery of a safe and potable water. The "multiple barrier" concept that relies on placing protective systems between the water consumer and actual as well as potential sources of contamination should be emphasized, with appropriate consideration for natural features (distance, dilution, geologic factors), man-made facilities (adequate waste treatment, water treatment, operational considerations), and conscientious surveillance by regulatory agencies (monitoring, inspection, certification). Investigation of three of the outbreaks that occurred in 1979 illustrates what can happen when these barriers are neglected. The outbreaks occurred in Bradford, Pa.; a state park near Lake Havasu City, Ariz., and a church camp near Brevard, N.C.

Bradford, Pa.

Bradford is located in northwestern Pennsylvania about 210 km (130 mi) northeast of Pittsburgh and some 97 km (60 mi) south of Buffalo, N.Y. It is situated in the midst of the Allegheny oil region and is an industrial center for producing and refining crude oil.

The water system serves a population of approximately 18 000. Three impoundments located within a few miles of the city serve as sources of supply. The impoundments and watersheds are wholly owned and protected by the city, and recreational use is prohibited. Chlorination is the only treatment provided; gas injection facilities are located immediately downstream from each impoundment. Transmission mains convey water from the impoundments to the distribution system, where the supplies converge. A number of service connections draw off each transmission main prior to their convergence.

An open finished water reservoir of 13-ML (3.5-mil gal) capacity provides storage and regulates pressure in the distribution system. Chlorination is not provided at this reservoir. Data for each raw water impoundment are given in Table 1.

The outbreak begins. A waterborne outbreak was suspected when fifteen residents reported within one week to the

Bradford Hospital for treatment of diarrheal illness. When tested, ten of the fifteen were stool-positive for *Giardia* cysts. Newspaper reports implicated the water supply and advised residents to boil their water. The advice was based upon reporters' discussions with state and city health officials and the Center for Disease Control (CDC) in Atlanta, Ga. The suspicion that the illness was related to the water supply probably arose from recent water quality problems.

Heavy rain in the Bradford area in July and August contributed to turbidity problems and resulted in numerous citizens' complaints of discolored and muddy water. This was compounded by public notification of failure to comply with the coliform maximum contaminant level (MCL) in August and September. The city was not required to report violations of the turbidity MCL because it had been granted a waiver by the Environmental Protection Agency (EPA). Records indicated that the reservoir at Marilla Brook had turbidity values in excess of 10 NTU on numerous occasions.

The bacteriological record appeared acceptable until August when three of twenty samples collected were reported to be positive for coliform bacteria (Table 2). Closer inspection of the record, however, showed that extensive time had elapsed between sample collection and analysis, casting doubt on the validity of all the results prior to the use of local laboratories in September.

TABLE 1
Characteristics of reservoir facilities in Bradford, Pa., 1979

Raw Water Impoundment	Drainage Area		Storage Capcity		Average Output		Construction Date
	km²	*sq mi*	*ML*	*mil gal*	*ML/day*	*mgd*	
Reservoir 2 (Gilbert Run)	533	206	765	202	5.3	1.4	1913
Reservoir 3 (Marilla Brook)			454	120	4.1	1.1	1900
Reservoir 5 (Hazelton Mill)			2574	680	14.4	3.8	1955

TABLE 2
Coliform sample results for Bradford, Pa., 1979

Month	Number of Samples	Number of Positive Samples	Number of Samples and Time Between Collection and Laboratory Analysis				
			Same Day	2 Days	3 Days	4 Days	5 Days
January	25	0			25		
February	20	0			20		
March	20	0			20		
April	25	0			25		
May	20	0			15	5	
June	20	0		4	5	11	
July	25	0		5		15	5
August	20	3				15	5
September*	26†	5	21		5		
October	24‡	2	24				
November	19	0	19				
December	20	0	20				

*Began using local laboratory
†Includes six check samples
‡Includes two check samples

Determining the cause. The Meadville regional office of the Pennsylvania Department of Environmental Resources invited EPA to assist in the investigation of the water system to isolate *Giardia* cysts from the water, identify the source of contamination, and propose corrective measures. Also, a team of epidemiological investigators was dispatched by the Pennsylvania Department of Health and was soon joined by a team from the Center for Disease Control.

Within ten days after the investigation was begun, cysts were isolated from the water system. A beaver trapped from a large lodge in the Gilbert Run reservoir was sacrificed for necropsy and a sample from the colon showed an abundance of *Giardia* cysts. At this point in the investigation the number of confirmed cases of giardiasis at the Bradford Hospital had increased to 180.

Precautionary measures taken at the outset of the investigation included a recommendation that consumers boil water for one minute and that plant operators increase chlorination to a concentration of sufficient strength to destroy *Giardia* cysts. Chlorine contact times were calculated for each source based on the flow in each main and the distance from the point of chlorine injection to the first service connection. These times were confirmed by increasing chlorine feed and clocking the amount of time it took for the increase to appear at the first service connection. The computed and observed times were in reasonable agreement, and chlorine doses were adjusted according to the most current information available for destruction of Giardia Cysts.

The chlorine contact time at the Gilbert Run reservoir was most critical at about 20 min, which required a chlorine dosage that would give a residual of 2.5 mg/L at the first service connection. Contact time at the Marilla Brook reservoir was 30 min, requiring a residual of 2 mg/L. The Hazelton Mill reservoir provided a contact time of more than 90 min, which permitted the use of a more reasonable chlorine dose. Feeding the entire system from Hazelton Mill was considered as a possibility but was rejected because inadequate pressure would prohibit service to all customers. However, after beaver lodges were found in both the Gilbert Run and Marilla Brooks reservoirs, the output from these sources was reduced and the deficit sustained by Hazelton Mill. Common problems noted at all of the chlorination facilities included antiquated equipment (a single chlorine cylinder was connected to the chlorinator, causing lapses in feed when empty cylinders were replaced so that if a 15-min change time at each facility occurred on the same day 250 kL [66 000 gal] of nonchlorinated water would be delivered to the system); inadequate chlorinator capacity; and an incorrect procedure for determining chlorine dose to achieve a residual in the distribution system. Chlorine residuals had been determined by collecting samples from a utility tap located a short distance from the injection point and adjusting the feed rate to attain 1 mg/L. This resulted in no residual in the system.

The city provided new chlorine feed equipment, which included manifolded cylinders with automatic switchover devices to improve the reliability of chlorination. Chlorination was the only protective barrier that could be employed in this situation, so uninterrupted, continuous disinfection was very important. Even though consumers had been instructed to boil water, this could not be considered an effective barrier because it was not known how diligently the boil water order was being followed in the community.

The Pennsylvania Game Commission estimated that six to eight beavers inhabited the lodge in Gilbert Run reservoir. It was suggested that they be trapped and moved out of the watershed; however, the commisson was concerned that the beavers would not survive in new surroundings that late in the year. Holding the beavers in captivity for release in the spring was then considered. Suitable holding facilities were not available and other problems associated with this

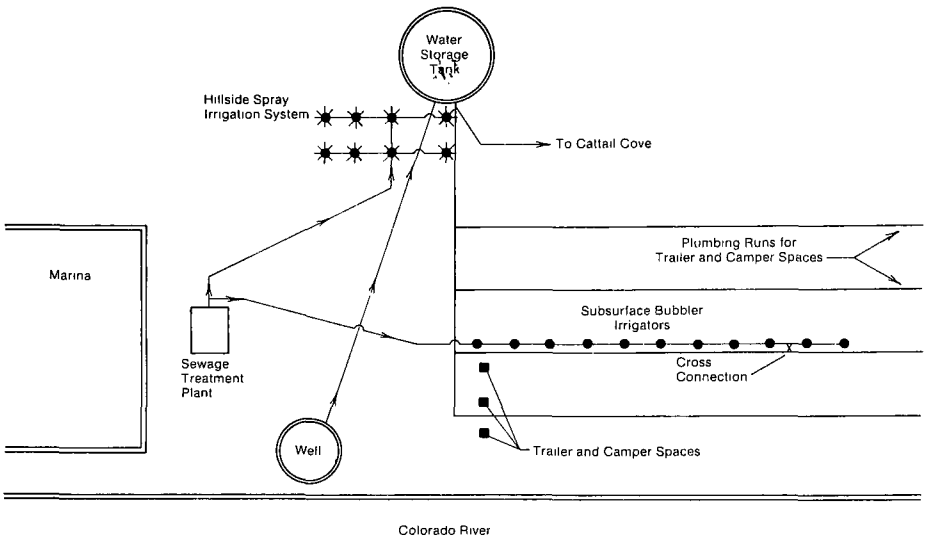

Figure 1. Water and sewage facilities for the Sand Point marina and campground

alternative made it unworkable. Eventually all the beaver in the Gilbert Run and Marilla Brook reservoirs and a few from the watershed of Hazelton Mill reservoir were trapped and sacrificed. Two beavers in Gilbert Run, one in Marilla Brook, and one in Hazelton Mill tested positive for *Giardia* cysts.

The number of giardiasis cases determined by positive stool examination at the Bradford Hospital eventually increased to 407. However, some local physicians began prescribing medication without the benefit of stool examination. Sales of drugs normally prescribed for giardiasis were recorded by city pharmacies as numbering 3487 during the six-week period beginning with the start of the investigation. The epidemiological study conducted by CDC and the state health department included a random household survey to identify occurrence and distribution of illness and collection of stool specimens to determine infectivity rate in residents of Bradford as well as a control community. The control community had little interaction with Bradford and used groundwater as a water supply source. The survey results showed that 5.4 percent of the Bradford residents had giardiasis-like illness (diarrhea for ≥ 10 days) with only 0.6 percent of the control community experiencing the same symptoms. Examination of stool specimens showed an infection rate of 16 percent for Bradford respondents (seventeen of 106 stools positive) and all 40 stools negative for the control community.[1] The stool infectivity rate applied to the Bradford population amounted to 2900 people affected.

The water quality problems that occurred before the outbreak generated numerous complaints, and public support was building for improvement of the water system. The additional inconvenience of boiling water combined with the noticeable odor created by increased chlorination fostered additional public pressure for action. The city directed a consulting engineering firm to begin a feasibility study for the provision of adequate treatment only eleven days after investigation of the outbreak began.

Rebuilding protective barriers. The Bradford water system is typical of many US systems faced with making improvements to comply with the National Interim Primary Drinking Water Regulations. In Pennsylvania alone there are 113 similar systems that rely on surface water as a source but do not have the facilities to reduce turbidity. From an energy conservation aspect, the Bradford system is very efficient, relying on raw water sources situated at sufficient elevation to maintain pressure throughout the distribution system without pumping. However, nearly two thirds of the system is unmetered, which is reflected in an average per capita demand of 1365 L/day (350 gpd) and in the size and cost of sewage collection and treatment facilities.

Breakdown occurred in all components of the protective systems in the multiple barrier concept. With information accumulating from waterborne outbreaks of giardiasis in California,[1] Washington,[2,3] Oregon,[1] and New Hampshire,[2,4,5] and other studies[6] implicating beaver as an intermediate host of the cyst that is transmitted to man, protective public health measures should be incorporated into the multiple barrier concept for certain water systems. Systems using surface water as a source of supply, with disinfection as the only means of treatment, should consider control of the beaver population in the watershed. Control measures may include periodic surveys to determine where lodges are being established, trapping during periods of the year when the beaver can be safely moved, and forestry practices that replace deciduous trees with coniferous species, thereby limiting the food supply along waterways and around reservoirs to discourage beaver habitation. These precautionary measures could be associated with natural and surveillance barriers, with both utility and regulatory agencies sharing the responsibility for implementation.

Other breakdowns that occurred were inadequate chlorination (man-made barrier) and ineffective microbiological monitoring (surveillance) which cast doubt on the validity of coliform results. The turbidity values prior to September were also questionable because the formazin standard was leaking. It is ironic that a waiver from compliance with the turbidity MCL was granted by EPA in this situation: such a waiver is predicated on qualifiers including demonstration that turbidity does not interfere with disinfection, prevent maintenance of an effective residual throughout the distribution system, or interfere with microbiological determinations.

State park—Lake Havasu City, Ariz.

Cattail Cove state park is located on the eastern bank of the Colorado River north of Parker Dam and south of Lake Havasu City. A recreational area located within the park is divided into two facilities, one operated by the state and an adjacent facility operated by concessionaires (Sand Point marina and campground). Sand Point, the larger of the two facilities, includes a store, comfort station, marina, and 170 spaces for campers and recreational vehicles.

The water system serving the recreational area is simple in design and operation. The source is a shallow well constructed in a diked area adjacent to the Colorado River, using bank storage or infiltrated river water. After chlorination, water is pumped to a hillside storage tank overlooking the two facilities. The Cattail Cove and Sand Point distri-

bution systems are fed by separate mains off a wye connection near the tank (Figure 1). Each facility has a sewage system consisting of a package plant with effluent disposal by irrigation.

Cause for concern. Disease control officials of the Arizona Department of Health Services requested assistance when they suspected a waterborne outbreak related either to swimming or water supply at the recreational area. Reports of illness began in June and continued through September. Most of the reports originated in California, which caused some problems in followup of cases. Of 74 cases investigated, four were stool positive for *Giardia* cysts. Information gathered by questionnaire suggested that drinking water at Sand Point was associated with illness.

Coliform sample results from the Sand Point water system showed two of two samples positive (TNTC and confluent growth) in June with followup check samples negative thirteen days later. (Two consecutive negative check samples were initiated on only one sampling point.) One of four samples collected in July was unsatisfactory (confluent growth) with no followup check sample, and one negative sample was collected in August. Samples from the state facility distribution system were all negative. Samples from the swimming areas showed coliform and fecal coliform results in excess of the single sample recommendation (200 fecal/1000 total) in August and September. After the Labor Day weekend beaches were posted with the warning that water quality did not meet standards and that swimming was therefore not recommended. While the preliminary epidemiological information implicated Sand Point drinking water, bacteriological data for the swimming areas also cast suspicion on water-related recreational activities. Suspicion increased when it was learned that the sewage plant serving Sand Point overflowed into the marina during a power failure in July. Overflow facilities had not been incorporated in the plant design.

Erratic evolution plagues system. The water and wastewater systems at Sand Point evolved over a period of six years under four different owners. An important feature unique to the Sand Point facility was the design of the sewage disposal system. The system was initially designed to dispose of the total sewage plant effluent through the use of subsurface bubbler irrigators. The design was based on 150 trailer or camper spaces utilizing 380 L/day/space (100 gpd/space) and 57 L/day/bubbler (15 gpd/bubbler), which required 1000 subsurface bubblers to irrigate an equal number of trees and shrubs. A surprising feature of the design was the use of the same type and color of pipe for the effluent irrigation system as for the potable water distribution system.

At some stage in the process of system development and changes in ownership, the installation of bubblers was halted and spray irrigation was added on an on-site hillside to aid in the disposal of effluent. Tree and shrub irrigation in the camping areas was then accomplished by drip irrigation using potable water with aboveground drip fittings submerged in pits formed around the bases of trees and shrubs. As the facility changed ownership and was expanded to accommodate additional trailer spaces, records or sketches were not kept to indicate where plumbing changes were made, and the extent of the bubbler system was not known. (The changes were made without the required approval by the health department.)

Conditions were optimized for creating a direct cross connection between the water system and the subsurface bubble-irrigation system. Each drip fitting submerged in a tree or shrub pit provided an entry point for contaminated water if subatmospheric pressure occurred in the water system.

To complicate the situation further, the location chosen for spray irrigation was on the side of a hill directly above the transmission main between the well and storage tank and the feed line to the campground. The feed lines to the campground were located in a gully at the base of the hill. Because the vegetation and soil on the hillside could not absorb all the effluent being applied, effluent ran off into the gully. To prevent drainage into the marina and beaches, small catchment areas were fashioned to retain the runoff in pools. These pools were located almost directly on top of the line carrying potable water. This presented another unusual situation.

When the well was developed, it was constructed in fill material placed to extend the bank into the river and form an infiltration gallery. Sufficient fill was excavated to set the well at a depth of 4.5 m (15 ft) and the material packed around the screen was to be selected and placed by specifications referenced on the engineering plan. It is assumed that this material was intended to be carefully selected, washed, and graded sand and gravel. However, local material was used in backfilling the gallery.

A raw water quality problem subsequently developed which is thought to be related to the use of material excavated from a local site that contains manganese. After chlorination, the water pumped from the well turned black in the transmission main to the storage tank, indicating the oxidation of manganese. The transmission main, rising about 45 m (150 ft) and running a length of approximately 300 m (1000 ft), served as an inclined settling tube that permitted an accumulation of black water which had to be bled off every three or four days and before weekends through a discharge port at the well and into the swimming area. This process discolored the river for about 30 min before the black water was diffused.

The unique situation thus presented was the possibility that while the transmission main to the storage tank was being bled, siphonage of sewage or drainage from the irrigated area could occur by a venturi effect through a crack or bad joint in the main, causing contamination of the beaches or affecting the water being infiltrated into the well.

Standard bacteriological samples were collected as a result of the concern with problems of the water and sewage systems and the swimming areas. A larger volume sample of raw water was collected from the well, using a filtering device containing three membrane pads in series with pore sizes of 5, 2, and 0.45 μm. Nineteen litres, or 190 times the volume of a standard bacteriological sample, were filtered through the pads for analysis of coliform and fecal coliform bacteria. (The pads were halved at the laboratory and processed separately for the analysis.) The large-volume sampling technique was used to detect low-level and, possibly, intermittent contamination.

Sample results showed no coliforms in the large-volume sample of raw water and no occurrence of contamination during bleed-off of the transmission main from the well to the storage tank. (The latter finding was also confirmed by a dye test.) Positive coliform and fecal coliform results were obtained on samples from the Sand Point distribution system in an area that served campers who had reported illnesses.

Discussion of the results with the departments of health services and state parks led to the agreement to close Sand Point marina and campground. By placing dye in the effluent pumping well of the sewage treatment plant, the northern regional office of the Department of Health Services in Flagstaff proved that a cross connection existed between the water system and the subsurface bubbler irrigation system. After dye was observed coming from the camp faucets, excavation located the cross connection. Extensive corrections were required to improve the water and sewage systems. Improvements also were required in surveillance activities, including prompt followup on positive coliform samples, timely reporting of results, and a review of the state regulations permitting installation of wastewater irrigation systems. Particular emphasis was placed on the necessity of color coding or use of dissimilar materials for water and wastewater piping.

Determination of the occurrence and

distribution of illness associated with this outbreak was difficult and time-consuming. Epidemiologists from the Department of Health Services were hampered in their investigative efforts by a number of factors, including (1) difficulty in locating visitors because of incomplete or erroneous identification entered on the camp register; (2) communication across state boundaries; (3) contact of visitors who might have been exposed five or six months before and would not remember; (4) selection of an appropriate sample population; (5) defining an incubation period where exposure to multiple pathogens could have occurred; (6) complications related to the possibility that the agent of hepatitis might have been involved; and (7) limited investigative resources.

Investigators eventually chose to interview a sample of the population that visited one of the two facilities on Labor Day weekend. A case was defined as a person who experienced a diarrheal illness during or within a three-week period after the visit. Results based on a telephone survey of 44 of 179 groups visiting Sand Point marina and campground and 14 of 42 groups who registered at the state facility showed that 57 (35.2 percent) of 162 campers from Sand Point were ill compared to only 6 (8.0 percent) of the 75 state park campers. The attack rate was significantly higher for Sand Point campers who consumed campsite water than for those who did not, and the incidence of illness was greatest in the vicinity of the cross connection. Approximately 6800 persons camped at Sand Point from June 1 through September. If water contamination occurred in this period, it is estimated that as many as 1900 persons may have become ill in this outbreak.[7]

Hazards lurk in noncommunity systems. The water supply serving Sand Point marina and campground is classified as a noncommunity system under the National Interim Primary Drinking Water Regulations. Current estimates place the total number of noncommunity systems at 250 000. Generally, this type of system has not received the same degree of attention from regulatory agencies as have the community water systems. The 59 000 community water systems have been given priority consideration in implementation of the Safe Drinking Water Act, which is not reassuring since they also suffer from lack of effective surveillance, as exemplified by the situation in Bradford, Pa. If such problems as those found at Sand Point marina and campground are common, regulatory surveillance will require substantial changes to control a potentially serious public health situation.

There was clearly a breakdown in regulatory surveillance for this system. Design criteria for reuse systems were not adequate, making review of plans and specifications ineffective. Procedures for inspections during construction and for changes after the system was in operation also were inadequate, permitting hazardous conditions to develop. Microbiological monitoring was not effective in followup procedures for positive samples.

The problems discussed that pertain to investigation of the outbreak do not fully describe the hazards observed in this situation. More than 100 potential cross connections existed with the drip irrigation system; drains for surface water could be mistaken for sewage hookups, allowing contamination of bathing areas; spray from the effluent irrigation system could drift over the camping area; valves on the water system were installed by bringing plumbing runs to the soil surface without using vaults or boxes; and potable water lines at the sewage dump station (for spaces without sewage hookups) were not properly protected.

Perhaps a more important concern is that noncommunity systems get the least regulatory attention and need the most. These water systems are frequently operated and maintained by untrained people and are beset with design, construction, and operational problems.

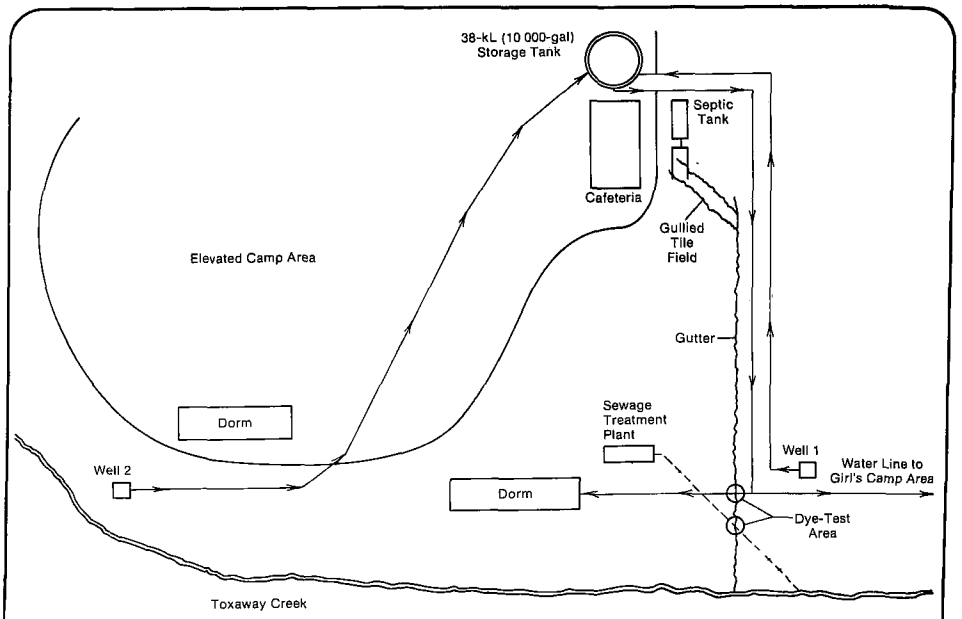

Figure 2. Water and sewage facilities at The Wilds camp

Church camp—Brevard, N.C.

A church camp named "The Wilds" is located in Pisgah National Forest in southwestern North Carolina. Religious groups use the camp for retreats held throughout the week during the summer months and over weekends during other seasons. Camp facilities include dormitories, assembly hall, cafeteria, and extensive recreational facilities.

The water system consists of two wells that supply untreated water to a storage tank from which it is pumped to the distribution system. The major water-using facilities discharge wastewater to a package sewage treatment plant. Septic tanks serve the few buildings inaccessible to the collection system (Figure 2).

Large number of illnesses closes camp. An epidemiologist with the North Carolina Department of Human Resources visited the camp the first weekend in November, after receiving reports of illness. It was determined that illnesses had been occuring in groups visiting the camp since August. A survey was conducted during the investigator's visit, with questionnaire results showing that 146 of 186 visitors were affected with vomiting and diarrhea within 24 hours of arrival. Stool and acute serum specimens were collected and arrangements made for collection of convalescent serum after a four-week interval. The camp was closed while the investigation evaluated food preparation and handling, general hygienic practices, and water supply and waste disposal facilities.

EPA participated in the investigation and evaluated water supply and waste disposal facilities. A large-volume water sample (1900 L [500 gal]) was collected for viral assay and 19-L (5-gal) samples for coliform and fecal coliform analyses were collected for each well using the filtering technique described in connection with the Arizona investigation. (Two standard bacteriological samples collected by the county sanitarian from the cafeteria kitchen the last two weekends in October were positive for coliforms.) Collection of 19-L (5-gal) bacteriological samples from each well on membrane filters showed coliform and fecal coliform contamination of well 1 but no

coliform colonies present on the membrane filter for well 2.

Sources of contamination that could conceivably affect the wells included the septic tanks and the effluent line from the sewage treatment plant. Well 1 was located about 15 m (50 ft) from the plant and the effluent line passed within 7.5 m (25 ft) of the well on its path to a discharge point in Toxaway Creek (Figure 2). The effluent line crossed a drainage ditch and was found to be damaged and leaking sewage in September. The area is underlain by limestone which can become fractured, making it possible for the well to be infiltrated by sewage effluent. (It was reported that an earth tremor occurred in the area in late July.) In addition, a short distance upstream from the location of the gutter crossing, a septic tank had been damaged. A truck had been driven over the site, rupturing the fiberglass tank. The tile field, which was located at the base of a steep slope, was eroded from surface drainage, and deep gullies had formed that drained into the gutter. It was therefore possible for sewage to enter the gutter from the ruptured septic tank or the gullied tile field. This, coupled with a water line crossing the drainage ditch and passing within immediate proximity of the well, was another possibility for contamination. Sewage flowing down the gutter could follow the path of the water line in the excavated and back-filled trench.

Both possibilities were tested by damming the gutter downstream from the water line and effluent line crossings and dosing the pool with fluorescein dye. The well was sampled unsuccessfully for fluorescein for three weeks. While fluorescein was apparently not charged into the well, it is conceivable that solution channels in the limestone strata became clogged and were sealed, preventing entry of the dye. Possibly other factors intervened. One septic tank that could have remotely influenced well 2 was also dye-tested with negative results.

The camp was permitted to reopen pending improvements which included installation of chlorination equipment on both wells, flushing and disinfection of the well system, and repairs to sewage disposal facilities. Well 1 was kept out of service during the opening weekend and no illness was noted among visitors.

Epidemiological survey data, reinforced by laboratory tests of medical specimens, determined that the agent responsible for this outbreak was a "Norwalk" type virus. Serological studies conducted by the Center for Disease Control showed that thirteen of fourteen symptomatic cases were seropositive for antibody titers against the Norwalk agent. Immune electron microscopy studies of stools from 35 ill volunteers failed to defect virus.[8] Detection of Norwalk agent in stool specimens collected during outbreaks has been very difficult. Volunteer studies under laboratory conditions have shown that about one half of those exposed to Norwalk agent excrete the particles in sufficient concentration to be detected by immune electron microscopy. Particles are not detected prior to onset of symptoms and are found in less than 20 percent of stools collected 72 hours or longer after onset of illness.[9]

Untreated groundwater supplies spell trouble. This outbreak is discussed here because it typifies many of those occuring in systems using groundwater as a source of supply without treatment. A review of the causes of waterborne outbreaks shows that for the period from 1971 through 1978, 107 of 224 reported outbreaks were caused by use of groundwater that was not chlorinated or not adequately treated. The outbreaks were responsible for a total of 17 391 illnesses. An etiologic agent was identified in fewer than half of the outbreaks, the remainder being characterized as acute gastrointestinal illnesses that were presumably caused by bacterial pathogens or enteric virus for which isolation and identification techniques were not available. It is not unusual for laboratories to culture specimens for only two bacterial pathogens, *Salmonella* and *Shigella*, and if they are not present, the causative agent is never determined.

There has been an improvement in the capability of some laboratories to detect other bacterial and viral pathogens that can be cultured. However, nonculturable viruses, which can be detected in stool specimens only by electron microscopy or through the use of stool material from infected individuals reacted with acute and convalescent serum to produce specific rises in antibody titer, have become increasingly associated with waterborne outbreaks in groundwater systems. It is typical of these outbreaks that investigators detect sources of sewage contamination in close proximity to wells but usually cannot demonstrate either through dye-testing or by sampling for microbiological indicators that contamination occurred. Viruses identified with these outbreaks are extremely small in size (in the range of 30–70 nm), which probably enables them to penetrate water-bearing aquifers whereas coliform organisms are trapped or die. Many factors interfere with dye-testing in groundwater situations, and the inability to demonstrate a positive test is not proof that the "likely event" did not occur.

Overall summary

A study of three waterborne disease outbreaks that occurred in 1979 shows that problems contributing to the occurrence of outbreaks can be identified and related to a breakdown of protective systems in the multiple barrier concept. Designers, utility personnel, and regulatory agencies share a responsibility for maintaining the integrity of the protective systems necessary to ensure the delivery of a safe and potable water.

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References

1. Waterborne Giardiasis—California, Colorado, Oregon, Pennsylvania. *Morbidity and Mortality Weekly Rep.*, 29:11. Center for Disease Control, PHS, DHEW, Atlanta, Ga. (Mar. 21, 1980).
2. Waterborne Giardiasis Outbreaks—Washington, New Hampshire. *Morbidity and Mortality Weekly Rep.*, 26:21 Center for Disease Control, PHS, DHEW, Atlanta, Ga (May 27, 1977).
3. Kirner, J.C.; Littler, J.D.; & Angelo, L.A. A Waterborne Outbreak of Giardiasis in Camas, Wash. *Jour. AWWA*, 70:1:35 (Jan. 1978).
4. Lippy, E.C. Tracing a Giardiasis Outbreak at Berlin, New Hampshire. *Jour. AWWA*, 70:9:512. (Sep. 1978).
5. Lopez, Carlos et al. Waterborne Giardiasis—A Community-Wide Outbreak of Disease and a High Rate of Asymptomatic Infection. *Am. Jour. Epidemiol.* (in press).
6. Davies, R.B. & Hibler, C. P. Animal Reservoirs and Cross Species Transmission of *Giardia*. Waterborne Transmission of *Giardiasis* Symposium, Natl. Tech. Information Service, Springfield, Va., EPA-600/9-79-001 (June 1979).
7. Starko, K. et al. Diarrhea in Campers (in preparation).
8. Finch, C. et al. Recurrent Gastroenteritis at a Camp in North Carolina (in preparation).
9. Blacklow, N.R.; Schreiber, D.S.; & Trier, J.S. *Viral Enteritis—Seminars in Infectious Disease*. Stratton Intercontinental Medical Book Corp. (1978).

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Chlorination to Prevent and Control Waterborne Diseases

Edwin C. Lippy

An analysis of statistics compiled on outbreaks of waterborne disease from 1946 to 1980 and from 1965 to 1982 showed that disinfection was not provided where it was needed and was not properly operated where it was provided. Chlorine concentration, contact time, pH, temperature, and interfering substances all must be evaluated to determine disinfection requirements. Use of reliable chlorination facilities and proper application of current technology can control and prevent waterborne diseases.

Two recent publications identified shortcomings in disinfection contributing to waterborne disease outbreaks. Statistics compiled for the period from 1946 to 1980 showed glaring deficiencies in disinfection practices.[1] The primary problems were that disinfection was not provided where it was needed and it was not properly operated where it was provided, both of which caused or contributed to nearly half of the outbreaks of waterborne disease in public water systems. The same shortcomings were identified when the 63 giardiasis outbreaks occurring in the period 1965-82 were analyzed.[2] Two-thirds, or 42, of these outbreaks were related to a failure to provide adequate or reliable treatment, with chlorination deficiencies identified as a primary cause.

This article reviews the factors that influence chlorine's effectiveness in inactivating bacteria, viruses, and protozoans for the purpose of preventing and controlling waterborne disease outbreaks. The factors reviewed are: (1) chlorine concentration, (2) contact time, (3) pH, (4) temperature, and (5) interfering substances. Each of these must be evaluated to determine disinfection capability under current operating conditions and to determine the adjustments necessary for coping with the challenges of pathogens.

Chlorine concentration and contact time

The product of chlorine concentration C (in milligrams per millilitre) and contact time T (in minutes) produces a CT relationship that is used in determining the requirements for inactivation of bacteria, viruses, and protozoans. Because CT relationships are developed through laboratory research under carefully controlled conditions, applying the data to a real world situation requires judgment. The CT values listed in Table 1 for viruses were developed by White[3] and also can be applied for inactivation of bacterial agents. The viral CT values are based on inactivation of Coxsackie A2 viruses, which are more resistant than poliovirus or any pathogenic vegetative bacteria implicated as causative agents in waterborne disease outbreaks. Values for protozoan CT were interpreted from research conducted by White,[3] Jarroll et al,[4] and Rice et al.[5] A rule of thumb can be derived from the CT relationships at pH values of 7-8, which are common to most water systems. Systems using groundwater as a source of supply should apply a minimum CT of 15-30 to provide for the inactivation of viruses and bacteria, which are generally the contaminants of concern; i.e., the size of viral particles permits easier penetration of strata overlying aquifers whereas protozoan cysts are trapped and retained (protozoan cysts are about 1000 times larger than virus particles), and bacteria are capable of reproducing in the environment whereas protozoan cysts are not. Systems using surface supplies, especially those located in areas where source water temperatures can be as low as 41°F (5°C), should apply a minimum CT of 100-150 for protozoan inactivation. At temperatures lower than 41°F (5°C), the CT requirements will increase. Figure 1 illustrates the CT requirements included in Table 1.

Once the CT value is known, the actual contact time is determined through evaluation of the treatment facility, and the required chlorine concentration is determined mathematically. Because several factors must be considered, determination of the actual contact time should be done carefully. A common mistake is using hydraulic displacement calculations to compute contact time. For example, a 1-mil gal (3.8-ML) rectangular storage tank, with inflow and outflow at opposite ends, provides 2.4 h of contact time when calculations consider the average daily plant output of 10

TABLE 1
CT *relationships for 99-percent inactivation of pathogenic agents*

	Viral CT		Protozoan CT		
pH	32-41°F (0-5°C)	50°F (10°C)	41°F (5°C)	59°F (15°C)	77°F (25°C)
6			80	25	15
7			100	35	15
7.0-7.5	12	8			
7.5-8.0	20	15			
8			150	50	15
8.0-8.5	30	20			
8.5-9.0	35	22			

Disinfection problems caused or contributed to more than half of the outbreaks of waterborne disease.

mgd (38 ML/d), but the contact time actually provided may be one fourth of the calculated value.[6] With an actual contact time of 36 min, a chlorine concentration based on the 2.4-h contact time would be insufficient. This difference in the calculated displacement or theoretical retention (contact) time and the actual retention (contact) time stems from short-circuiting and dead spaces common to both rectangular and circular storage tanks. The use of the average daily plant output can also lead to errors. Plant output varies with customer demands. Peak daily demands for some systems can be twice the average daily plant output. If this is the case, the contact time is underestimated by an additional 50 percent. If the chlorinator is not paced to respond to changes in plant output, fluctuation in demand must be taken into consideration when evaluating contact time and chlorine dosage requirements.

The preferred method of determining contact time is to conduct a tracer test. One simple and quick method is to increase chlorine feed and determine chlorine residual concentrations in the basin effluent. The initial increase in concentration signals the flow-through time for a fraction of the plant output, and subsequent samples will reflect a steady rise in chlorine residual until the incremental dosage has been accounted for. The mixed-flow and dead space components of basin hydraulics can also be estimated through application of the tracer methods discussed by Hudson.[7] Another simple and quick method for tracer tests is to cease fluoride feed and read the decrease in fluoride concentration over time. The initial decrease in fluoride concentration signals the flow-through time for a fraction of plant output and represents the contact time provided for a portion of the basin volume. The advantage of using chlorine and fluoride as tracers is that there is no need to introduce other dyes, salts, or chemicals into the water system. Use of other tracers may require special permission because of concern about toxicity. Decreasing the concentration of fluoride or increasing the concentration of chlorine for a short time should not cause objections to conducting the test. The elapsed time between the increase or decrease of chemical dosage and the initial corresponding change in the basin effluent represents the contact time and should be used in the *CT* relationship to calculate the concentration of chlorine that will be required to achieve a microbiocidal residual. The *CT* relationships are based on maintaining the chlorine concentration for the entire contact time; therefore, the dosage must be adjusted accordingly to compensate for chlorine demand and dissipation.

Tracer tests are normally not required where transmission or distribution mains are depended on for contact time. The mains are pressurized and, under full-flow conditions, the hydraulic characteristics approximate plug flow. With plug flow the theoretical and actual contact times are equivalent. However, they can be easily checked by increasing the chlorine dosage and measuring for the increase in residual at an appropriate point downstream. Table 2 is a ready reference for computing contact time in transmission and distribution mains. By dividing the pipe capacity (adjusted for available length) by the plant output or flow, the contact time can be determined. For example, at an output of 1 mgd (3.8 ML/d) with 1000 ft (300 m) of 24-in. (600-mm) pipe, contact time = 34 min.

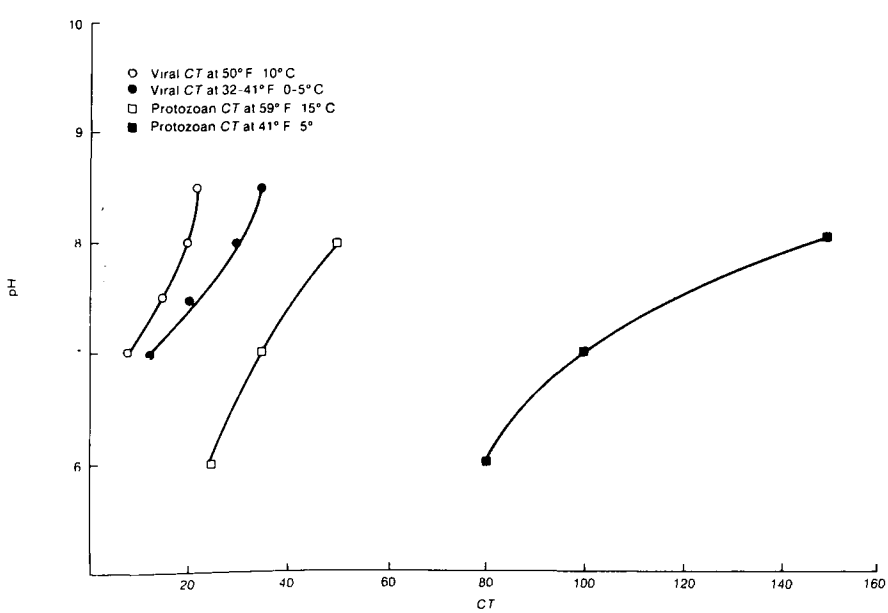

Figure 1. *CT* relationships for 99-percent inactivation of pathogenic agents

pH and temperature

The effectiveness of chlorine as a disinfectant is influenced by the pH and temperature of the water being dosed. The pH determines the amount of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) in solution. HOCl is the predominant form at lower pHs (6–7.5) and OCl^- is the predominant form at higher pH values of (8–10). At pH 7 and 32°F (0°C), the dissociation of chlorine produces 87.04 percent HOCl and 12.96 percent OCl^-; at pH 9, it produces 6.29 percent HOCl and 93.71 percent OCl^-.[8] It is important to know the dissociated forms of chlorine present at various pH values because HOCl is the form that possesses the destructive power as a microbiocide. According to White, "the germicidal efficiency of HOCl is due to the relative ease with which it can penetrate cell walls. This penetration is comparable to that of water, and can be attributed to both its modest size (low molecular weight) and its electrical neutrality (absence of an electrical charge)." White states that the hypochlorite ion "is a relatively poor disinfectant because of its inability to diffuse through the cell wall of microorganisms due to the negative electrical charge." Research has shown that HOCl is 80 times more effective than OCl^- in the inactivation of *Escherichia coli* and about

HOCl is the form of chlorine that possesses the destructive power as a microbiocide.

150 times more effective for cysts of *Entamoeba histolytica*.[3]

The key point to remember in the evaluation of chlorination is that the *CT* relationship increases with pH. Contact time, or the *T* portion of the *CT* relationship, is controlled by structural facilities such as contact basins, storage tanks, or transmission mains whose size cannot be readily adjusted to compensate for a needed increase in *CT*. Therefore, the only immediate alternative is to increase chlorine concentration. If pH is such that OCl^- is the predominant form, it may be necessary to lower the pH so that HOCl is the predominant form. Adjustment of pH should be considered when the chlorine requirement becomes excessive and, perhaps, objectionable.

In water, chlorine hydrolyzes rapidly—occurring in tenths of a second at 65°F (18°C) and in a few seconds at 32°F (0°C). Although temperature has little effect on chlorine's solubility, chlorine's reaction with microorganisms is affected. *CT* relationships are inversely related to water temperature, and as it decreases, the *CT* increases (Table 1). Therefore, temperature must be considered when selecting a bactericidal, viricidal, or cysticidal chlorine dosage.

Interferences

Chemical interference. When chlorine is introduced into water, it not only reacts to form microbiocides but also reacts with other substances to form organic and inorganic compounds that have little or no disinfection capability. The reaction of chlorine with organic and inorganic substances constitutes chemical interference with disinfection through consuming a part of, or all of, the applied chlorine—leaving little or no chlorine to react as a disinfectant. Organic and inorganic forms of nitrogen are among the compounds that significantly interfere with the chlorination of surface water. Hydrogen sulfide, iron, and manganese are commonly found in groundwaters and interfere with disinfection by combining with chlorine to form sulfates, ferric hydroxide, and manganese dioxide, respectively. The significance of this interference is exemplified by the requirement of 8.32 mg/L of chlorine to oxidize 1 mg/L of hydrogen sulfide.[3] In fact, the ability of chlorine to oxidize hydrogen sulfide, iron, and manganese is used to advantage as a treatment technique and is employed at some water plants for removal of these substances.

Physical interference. This type of interference occurs when microorganisms become entrapped within the matrix of particulate matter and are shielded from the effects of disinfection. Research[9-14] indicates that the protective effect of shielding by inorganic particulates is minimal and that inorganic particulates are of little consequence as physical interfering substances. Organic particulates such as those associated with sewage effluent solids do, however, afford protection and permit survival of microorganisms after long chlorine contact periods (60 min). A recent study by LeChevallier et al[15] on the survival of coliform organisms in the presence of turbidity indicated that after 1 h of contact time there was a 99.5-percent reduction in the number of organisms at a concentration of 6 ntu or less and an approximately 20-percent reduction at a concentration of 8–13 ntu. The study showed that turbidity control, to at least the 1 and 5 ntu concentrations as required by US Environmental Protection Agency regulations, is justified. Although more research is needed to specifically define the degree of physical interference resulting from turbidity and particulates, common sense dictates that where sewage discharges affect raw water quality, turbidity should be reduced by appropriate treatment (e.g., mixing, coagulation, flocculation, settling, filtration) for chlorination to be effective. If chlorination is the only treatment, it must be adjusted to compensate for the physical interference of turbidity.

Example of optimizing chlorination

An example is provided to illustrate the procedure that can be used in optimizing chlorination to cope with a pathogen challenge. Assume that a community uses an impoundment on a mountain stream as a source of supply. There are no sources of human contamination in the watershed. Chlorination is provided at the impoundment, with contact time occurring in 5000 ft (1500 m) of 24-in. (600-mm) transmission main. The average daily demand is 2 mgd (7.6 ML/d), which is increased by 50 percent during peak periods. The water temperature is 41°F (5°C), pH is 7, and turbidity is 1 ntu. Research data indicate the *CT* is 100 to offset a particular pathogen challenge. The chlorine dosage requirement is calculated for peak output, and it has been assumed that the chlorinator feeds in proportion to flow.

Peak output: 2 mgd × 150% = 3 mgd
Contact time: (2350 gal*/100 ft) × (5000 ft/3 mgd) = 56 min
Chlorine demand: test with chlorine comparator kit yields a demand of 0.5 mg/L at 56 min

*From Table 2

TABLE 2
Fluid capacity of pipes

Pipe Diameter—*d*		Capacity*	
in.	*mm*	*gal/100 ft*	*L/30 m*
3	80	37	140
4	100	65	246
6	150	147	556
8	200	261	988
10	250	408	1 544
12	300	588	2 226
14	350	800	3 028
16	400	1044	3 952
18	450	1322	5 004
20	500	1632	6 178
24	600	2350	8 896
30	750	3672	13 899
36	900	5288	20 017
42	1050	7197	27 244
48	1200	9400	35 583

*$4.08d^2$

Chlorination, properly applied, could have prevented the outbreaks.

Chlorine dosage requirement: (CT/T) + demand
$(100/56) + 0.5$
$= 1.8 + 0.5$
$= 2.3$ mg/L

The calculations account for all factors affecting chlorination, except for extrapolating the CT relationship from laboratory research data to applied use. An increased dosage of 0.5–1.0 mg/L would not be unreasonable. For this example, a dosage of 3 mg/L might be chosen to account for unknown variables in extrapolation. Increasing the dosage to 3 mg/L has the effect of adjusting the CT relationship by 40 percent (i.e., $1.8 + 0.7 \times 56 = 140$). The free chlorine residual that is produced, 2.5 mg/L after 56 min contact time, should be confirmed by monitoring. Microbiological monitoring for the pathogenic agent may be performed to confirm the efficacy of chlorination and account for extrapolation. If sampling or analytical methods are not available for pathogen monitoring, chlorination efficacy could be gauged by using methods that determine inactivation of the total bacterial population.

The above example assumes a flow-proportioning chlorinator that automatically adjusts dosage in response to changes in plant output. If a constant-feed chlorinator is used, several adjustments of dosage are required throughout the day. For example, between the hours of 10:00 p.m. and 6:00 a.m. customer demands decrease, and plant output responds accordingly. If demand decreases to 50 percent of the average, the chlorine residual is going to increase to objectionable concentrations unless the dosage is adjusted. With a 50-percent decrease in average output, or 1 mgd (3.8 ML/d), and the chlorinator adjusted to maintain the 3-mg/L dosage calculated for 3 mgd (11 ML/d), the chlorine residual will increase dramatically during low-demand hours to 8.5 mg/L. In this example, the resulting chlorine residual would likely cause widespread consumer complaints about tastes and odors.

Equally as important as providing the correct dosage is assuring that reliable chlorination is in operation 24 hours a day. Reliability demands dual cylinder feed, auxiliary power, standby equipment and spare parts, and maintenance of records documenting operation. Dual cylinder feed is necessary to ensure continuous dosage so that if one cylinder becomes depleted, the fully-charged one automatically comes on-line. A dual cylinder hookup also is needed to ensure continuous feed during cylinder replacement. Changing an empty cylinder for a full cylinder takes about 30 min, and if a dual hookup is not provided, unchlorinated water will enter the distribution network. This means that for a plant output of 1 mgd (3.8 ML/d), 21 000 gal (79 493 L) of unchlorinated water would be distributed. Auxiliary power is necessary to sustain operation during power outages. A standby chlorinator and spare parts are needed for uninterrupted operation during maintenance and repairs. Records to substantiate reliability in operation, including pounds of chemical used, plant output on a daily basis, and chlorine residual measurements, are also required. Current technology to ensure reliability in chlorination utilizes loop-controlled feed with remote sensing and automatic adjustment of dosage to a predetermined residual. The residual is continuously analyzed and recorded on a circular or strip chart, and an alarm system can be installed to warn of low residuals. The continuous record substantiates chlorination, or lack of it, and is a very important factor in verification of proper disinfection.

The factors that influence the effectiveness of chlorination were evaluated and the required CT successfully applied, halting transmission of waterborne disease. If adequate chlorination facilities had been in place and current technology properly applied to cope with the challenge of pathogenic agents, the outbreaks could have been prevented.

References

1. LIPPY, E.C. & WALTRIP, S.C. Waterborne Disease Outbreaks, 1946–1980: A Thirty-Five Year Perspective. *Jour. AWWA*, 76:2:60 (Feb. 1984).
2. LIPPY, E.C. & LOGSDON, G.S. Where Does Waterborne Giardiasis Occur, and Why? Proc. 1984 Specialty Conf. on Envir. Engrg. ASCE, New York (1984).
3. WHITE, G.C. *Handbook of Chlorination*, Van Nostrand Reinhold Co., New York (1972).
4. JARROLL, E.L.; BINGHAM, A.K.; & MEYER, E.A. Effect of Chlorine on *Giardia lamblia* Cyst Viability. *Appl. & Environ. Microbiol.*, 41:483 (1981).
5. RICE, E.W.; HOFF, J.C.; & SCHAEFER, F.W. Inactivation of *Giardia* Cysts by Chlorine. *Appl. & Environ. Microbiol.*, 41:250 (1982).
6. LIPPY, E.C. Tracing a Giardiasis Outbreak at Berlin, N.H. *Jour. AWWA*, 70:9:512 (Sept. 1978).
7. HUDSON, H.E. JR. *Water Clarification Processes: Practical Design and Evaluation.* Van Nostrand Reinhold Co., New York (1981).
8. WHITE, G.C. *Disinfection of Wastewater and Water for Reuse.* Van Nostrand Reinhold Co., New York (1978).
9. BOARDMAN, G.D. Protection of Waterborne Viruses by Virtue of Their Affiliation With Particulate Matter. Doctoral dissertation, Univ. of Maine (1976).
10. HOFF, J.C. The Relationship of Turbidity to Disinfection of Potable Water. *Evaluation of the Microbiology Standards for Drinking Water* (C.W. Hendricks, editor). USEPA-570/9-78-006 (1978).
11. BOYCE, D.S.; SPROUL, O.J.; & BUCK, C.E. The Effect of Bentonite Clay on Ozone Disinfection of Bacteria and Viruses in Water. *Water Res.*, 759 (1981).
12. WALSH, D.S.; BUCK, C.E.; & SPROUL, O.J. Ozone Inactivation of Floc Associated Viruses and Bacteria. *Jour. Envir. Engrg. Div.—ASCE*, 106:761 (1980).
13. STAGG, C.H.; WALLIS, C.; & WARD, C.J. Inactivation of Clay-Associated Bacteriophage MS-2 by Chlorine. *Appl. & Environ. Microbiol.*, 33:385 (1977).
14. SCARPINO, P.W. Effect of Particulates on Disinfection of Enteroviruses in Water by Chlorine Dioxide. USEPA 600/2-79-054 (1979).
15. LECHEVALLIER, M.W.; EVANS, T.M.; & SEIDLER, R.J. Effect of Turbidity on Chlorination Efficiency and Bacterial Persistence in Drinking Water. *Appl. & Environ. Microbiol.*, 42:159 (1981).

IV. Sampling and Analytical Methods

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SMALL ROUND STRUCTURED VIRUSES: THE NORWALK FAMILY OF AGENTS

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SRSVs of the Norwalk family have remained among the most difficult viral agents to detect, characterise and understand. Members of this family share an asymmetrical structured morphology when visualised directly in the electron microscope, but they are antigenically distinct when examined using immune electromicroscopy (IEM). None of these agents has been successfully cultivated, few have been adequately purified so that either polyclonal antisera or monoclonal antibodies can be prepared, few are visible with ease by EM, and nearly all assays developed to detect the antigen of these agents require human sera and stool as basic reagents. Since exposure to these agents is common, and since many of these agents may be antigenically related,[1] antibody responses in humans may not be restricted to the infecting particle. Consequently, relatively few of the convalescent human antisera obtained, even from volunteers, can be used as specific reference reagents.

The literature on the Norwalk family of agents is filled with a colourful variety of names of particles found in a single epidemic. These agents, of which nearly a dozen have been identified, are often unsuitable as references because samples from the key outbreaks have been exhausted in characterising the new particle against other reference agents. Only where volunteer studies have been conducted to amplify the volume of these reagents has the particle been further characterised and assays set up for its detection.[2-4]

In recent years, there have been more reviews written about these novel agents of viral gastroenteritis than substantive advances in our understanding of these agents.[5-11] Consequently, rather than review the history of each of these particles again, I present an approach used at CDC to assess where we would like to go in the future in combining epidemiological and laboratory studies. In the past, epidemiological investigations have been key to identifying novel diarrhoea agents and establishing their role as pathogens in man. Improvements in the investigation of future outbreaks—paying special attention to more voluminous specimen collection, more basic virology and extended laboratory collaborations—may help improve our understanding of the epidemiology of disease caused by these agents and provide the basic diagnostic reagents required to achieve major breakthroughs in understanding these viruses.

Epidemiological investigations as a key to discovery of new agents

In 1986, we reviewed the activities of the viral gastroenteritis group at CDC to asess the role of epidemic investigations in discovering new viral agents and in establishing their causal role in gastroenteritis. Two of the SRSVs recognised to be causes of diarrhoea—the Norwalk agent[12] and the Snow Mountain agent[13]—were each identified during the investigation of outbreaks of diarrhoea of undetermined aetiology by CDC epidemiologists. Human specimens from each of these outbreaks were subsequently used to establish the identity and causative role of the virus using techniques of immune electron-microscopy and to prepare inocula for both primate and human volunteer challenges. Had either of these agents been seen in a single individual, establishing their role as the cause of diarrhoea would have been impossible. However, the coincidence of finding particles in many of the outbreak victims but not in controls, combined with the determination of seroconversion to the particle by IEM among victims but not controls, helped link the agent in the chain of causality. Specimens from volunteer studies with the Norwalk agent were used by Greenberg and Kapikian to develop an RIA to detect antigen and a blocking assay to measure antibody titre.[3] Use of this assay with specimens from the CDC collection of unsolved outbreaks led to the recognition that the Norwalk agent was a prime cause of about 25% of the outbreaks of non-bacterial gastroenteritis in the USA. This RIA assay was adapted by Gary for use with biotin-avidin, leading to a sensitive and non-radioactive method to detect antigen and seroconversion to the Norwalk agent.[3,14] These assays have remained the key diagnostic tests in our laboratory. At the same time, the infrequency of detecting SRSVs by direct EM led over the years to the near abandonment of the use of electronmicroscopic methods in our laboratory. Further seroepidemiological studies with the Norwalk agent established that Bangladeshi children and American adults had a high prevalence of antibody, indicating that the Norwalk virus could be a cause of sporadic diarrhoea as well.[15]

Investigations by other groups have led to the identification of a variety of other particles, including the Hawaii agent, Montgomery County agent and Marin County agent in the USA; the Taunton, Moorcroft and Barnett agents in the UK; the Paramatta agent in Australia; the Sapporo and Otofuke agents in Japan as well as a group of other similar particles differentiated by IEM in Japan.[16] The wide antigenic diversity of this group, the inability to compare agents and to refine nomenclature, and the difficulty in obtaining quantities of the virus from outbreak specimens or passage in animals or cell culture have slowed progress in the field.

Review of outbreak investigations

We have reviewed results from the last 100 outbreaks investigated by CDC staff where stools and paired sera have been collected and assayed using the biotin-avidin test for the Norwalk agent (Fig. 1). Our intent was to confirm previous observations on the prevalence of Norwalk-associated outbreaks in this collection while looking at outbreaks that were still without an aetiological diagnosis to see if the causative agent could be found. An outbreak was defined as being associated with the Norwalk agent if, from six or more paired sera collected from victims of the outbreak, 50% or more demonstrated seroconversion defined as a four-fold rise in titre in the biotin-avidin assay. We found that the 20–30% positive rate for Norwalk-associated outbreaks observed in earlier studies[17,18] has been

maintained recently. Frequency distribution was not made up of two clear and distinct peaks of Norwalk-positive and Norwalk-negative outbreaks. Rather, rates of seroconversion represented a continuum with definite positive and negative outbreaks as well as a clear middle ground distinguished by outbreaks having a low percentage of seroconversions (10–49%) in which convalescent titres rose only to intermediate values (e.g. 800 or 1600). The intermediate group might represent infection with a virus that antigenically cross-reacted with the Norwalk agent, that produced an anamnestic response to a related antigen, or was ill-defined or non-specific due to an insensitive assay system.

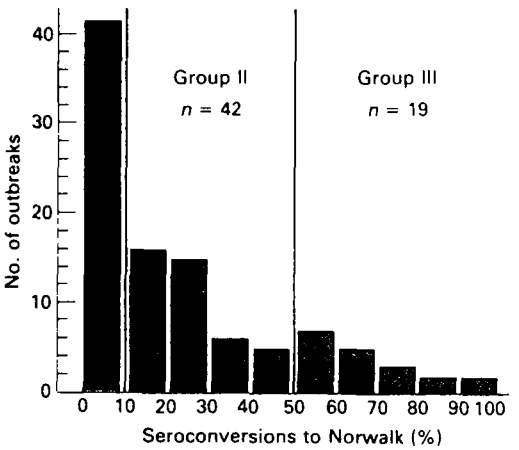

Figure 1: Serological analysis of outbreaks of Norwalk virus infection (n = 100), 1985–1988.

Selected outbreaks in the intermediate group (Group II) have been examined carefully and in collaboration with other investigators in an effort to identify other viral agents. Panels of specimens from 10–20 outbreaks have been shared with other investigators including Dolin and Madore (Rochester) to look for Snow Mountain agent, Blacklow and Herrmann (Worcester) to assay for astrovirus, Cubitt (London) to look for SRSVs using an antigen detection system still being developed, and Lewis (Taunton) to look by IEM for three specific Norwalk-like particles such as the Taunton agent. Some of our initial results are presented below.

Taunton agent

Paired sera from 10 outbreaks from the intermediate group were tested using an immune capture assay to look for a serological rise to the Taunton agent.[19] Paired sera from one outbreak in a nursing home in Florida proved positive for the Taunton particle, so 13 additional paired sera from this outbreak were examined. Of these, 11 showed a significant and diagnostic rise to the Taunton agent, identifying this to be the first such outbreak identified in the USA. These paired sera represent a reference resource to look further for the Taunton particle in future outbreaks.

Snow Mountain agent

In January 1988, we investigated an outbreak of diarrhoea aboard a British cruise ship that had come into US territorial waters. A small particle had been seen in an outbreak on a previous cruise. More than 20% of the passengers developed diarrhoea in the two weeks before the investigation. Paired sera and stools were negative for the Norwalk agent by both antibody and antigen detection assays. The sera did respond to the Snow Mountain agent with four of 19 passengers showing seroconversion and 11 of 19 demonstrating high acute titres. One faecal specimen had a visible particle which was negative in an assay for Norwalk or Snow Mountain antigen. This outbreak appears to be due to a small particle antigenically related to the Snow Mountain agent.

Calicivirus

In February 1988, an outbreak of gastroenteritis occurred among children in an day care centre in Sydney, Australia. Caliciviruses were seen in small quantity from several stool specimens taken from the children in the outbreak. These stool specimens and paired sera from patients were negative for the Norwalk antigen by biotin-avidin assays. They were examined in an enzyme immunoassay for calicivirus[20] and found to be positive, making this the first outbreak involving calicivirus identified in Australia.

Detection of SRSV antigen

Faecal specimens from 23 outbreaks in the CDC collection were tested for SRSV antigen using a biotin-avidin assay developed by Cubitt. This

assay recognises a variety of SRSVs, including the Norwalk agent, although the exact specificities of the capture and detection antibodies are unknown. The assay was able to determine between outbreaks where many specimens were positive and other outbreaks where all specimens were negative, a clustering which could not occur by chance alone. While the assay remains to be further characterised, incorporation of biotinylated human convalescent sera from other non-Norwalk outbreaks into assays similar to the biotin-avidin test for Norwalk antigen may provide a novel way to detect other SRSVs.

The new challenge in epidemic investigations

Our experience suggests that the investigation of new outbreaks of undiagnosed diarrhoea may be particularly valuable for identifying new agents and collecting reference reagents when the outbreaks are recurrent, ongoing, or located in enclosed environments such as aboard cruise ships, in nursing homes, or in institutional settings. Investigations in these settings permit collection of large volume stools repeatedly from patients who are acutely ill and where both acute and convalescent sera can be drawn in large volume. Convalescent sera which have particularly high titre have encouraged us to go back to patients and collect a one unit bleed to use for future reference work. On the basis of our experience with both the Snow Mountain agent and Norwalk agent we believe that outbreak investigations can provide adequate enriched specimens suitable for virus purification, preparation of inocula for challenge, experiments with cultivation and attempts at blind cloning. A major advance in understanding the virology of these organisms will be essential to advance our understanding of the true importance of these agents. In the interim, enzyme immunoassays appear to provide a method to screen outbreak specimens for the Norwalk agent, caliciviruses, Snow Mountain agent and other unspecified SRSV antigens.

References

1. Cubitt WD, Blacklow NR, Herrmann JE, Nowak NA, Nakata S, Chiba S (1987) Antigenic relationships between human caliciviruses and Norwalk virus. J Infect Dis 156: 806–814.
2. Dolin R, Levy AG, Wyatt RG, Thornhill TS, Gardner JD (1975) Viral gastroenteritis induced by the Hawaii agent: jejunal histopathology and seroresponse. Am J Med 59: 761–769.
3. Greenberg HB, Wyatt RG, Valdesuso J *et al.* (1978) Solid-phase microtiter radioimmunoassay for detection of the Norwalk strain of acute nonbacterial, epidemic gastroenteritis virus and its antibodies. J Med Virol 2: 97–108.
4. Dolin R, Roessner KD, Treanor JT *et al.* (1986) Radioimmunoassay for detection of the Snow Mountain agent. J Med Virol 19: 11–18.
5. Blacklow NR, Cukor G (1981) Viral gastroenteritis. N Engl J Med 304: 397–406.
6. Caul EO. Small round human fecal viruses. In: Pattison JR (ed) Parvoviruses and Human Disease, pp.140–153. Boca Raton: CRC Press.
7. Ciba Foundation. Novel diarrhoea viruses. Chichester: John Wiley, 1987. (Symposium no. 128).
8. Cukor G, Blacklow NR (1984) Human viral gastroenteritis. Microbiol Rev 48: 157–179.
9. Dolin R, Treanor JJ, Madore HP (1987) Novel agents of viral enteritis in humans. J Infect Dis 155: 365–376.
10. Kapikian AZ, Chanock RM (1985) Norwalk group of viruses. In: Fields BN (ed) Virology, pp.1495–1517. New York: Raven Press.
11. Madeley CR (1987) Viruses associated with acute diarrhoeal disease. In: Zuckerman AJ, Banatvala JE, Pattison JR (ed) Principles and Practice of Clinical Virology, pp.159–196. New York: John Wiley.
12. Adler I, Zickl R (1969) Winter vomiting disease. J Infect Dis 119: 668–673.
13. Morens DM, Zweighaft RM, Vernon TM *et al.* (1979) A waterborne outbreak of gastroenteritis with secondary person-to-person spread: association with a viral agent. Lancet I: 964–966.
14. Gary GW, Kaplan JE, Stine SE, Anderson LJ (1985) Detection of Norwalk virus antibodies and antigen with a biotin-avidin immunoassay. J Clin Microbiol 22: 274–278.
15. Black RE, Greenberg HB, Kapikian AZ *et al.* (1982) Acquisition of serum antibody to Norwalk virus and rotavirus and relation to diarrhea in a longitudinal study of young children in rural Bangladesh. J Infect Dis 145: 483–489.
16. Ando T, Sekine S, Hayaski Y *et al.* (1987) Role of small round viruses in foodborne gastroenteritis occurring in winter: Outbreaks in metropolitan Tokyo and epidemiologic research. (Jap). Jap J Food Microbiol 4: 103–134.
17. Greenberg HB, Valdesuso J, Yolken RH *et al.* (1979) Role of Norwalk virus in outbreaks of nonbacterial gastroenteritis. J Infect Dis 139: 564–568.
18. Kaplan JE, Gary GW, Baron RC *et al.* (1982) Epidemiology of Norwalk gastroenteritis and the role of Norwalk virus in outbreaks of acute nonbacterial gastroenteritis. Ann Intern Med 96: 756–761.
19. Lewis DC, Lightfoot NF, Pether JVS (1988) Solid phase immune electron microscopy with human immunoglobulin M for serotyping of Norwalk-like viruses. J Clin Microbiol 26: 938–942.
20. Nakata S, Estes MK, Chiba S (1988) Detection of human calicivirus antigen and antibody by enyzme-linked immunosorbent assays. J Clin Microbiol 26: 2001–2005.

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DNA Hybridization Probe for Clinical Diagnosis of *Entamoeba histolytica*

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As an alternative to microscopic identification of *Entamoeba histolytica* parasites isolated from stool, a sensitive and species-specific DNA hybridization probe was made for rapid diagnosis of *E. histolytica* parasites in clinical samples directly applied to nylon membranes. The DNA hybridization probe was made by screening a genomic library of a virulent HM-1:IMSS strain of *E. histolytica* to detect recombinant plasmids containing highly repeated parasite DNA sequences. Four plasmid clones that reacted across *Entamoeba* species coded for highly repeated rRNA genes of *E. histolytica*. Four other plasmid clones were *E. histolytica* specific in that they bound to four axenized and nine xenic strains of *E. histolytica* but did not recognize closely related *E. histolytica*-like Laredo, *Entamoeba moshkovskii*, or *Entamoeba invadens* parasites. The diagnostic clones detected as few as eight cultured amoebae and did not distinguish between pathogenic and nonpathogenic zymodemes of *E. histolytica*. The diagnostic clones were sequenced and contained 145-base-pair sequences which appear to be tandemly repeated in the genome. No stable transcript which is homologous to the diagnostic DNA was detected. In a study of stool samples from Mexico City shown by microscopy to contain *E. histolytica*, *Entamoeba coli*, *Giardia lamblia*, *Endolimax nana*, *Trichuris trichiuria*, and *Chilomastix mesnili* parasites, the DNA hybridization probe demonstrated a sensitivity of 1.0 and a specificity of 0.93. We conclude that the DNA hybridization probe can be used for rapid and accurate diagnosis of *E. histolytica* parasites.

Entamoeba histolytica is an enteric protozoan parasite which causes amoebic dysentery in humans (12, 26). The most common infection with *E. histolytica* is asymptomatic, in which the amoeboid forms of the parasite, called trophozoites, reside in the lumen of the colon and transform to cysts, which are passed in the feces. Such asymptomatic infections with *E. histolytica* are frequent among homosexual men in Western countries (1). However, *E. histolytica* is a major cause of morbidity and mortality in developing countries such as Mexico and India, because the trophozoites may invade the colonic mucosa and cause dysentery or liver abscesses or both.

Presently, infections with *E. histolytica* are diagnosed by light microscopy identification of trophozoites or of cysts separated from stool by dilution, filtration, and flotation during centrifugation (5). These methods are time consuming and require extensive experience to assure accuracy of morphological identification. Past or present invasive amoebiasis may be suggested by the identification of antiamoebic antibodies in patient serum (24).

In this paper, recombinant DNA methodologies previously used to create sensitive and specific diagnostic probes for *Plasmodium falciparum* and *Onchocerca volvulus* parasites (3, 21) are applied to *E. histolytica*. We show that the DNA hybridization probe binds to highly repeated and species-specific DNA sequences in *E. histolytica* and that the probe can be used to identify parasites directly in patient stool samples.

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MATERIALS AND METHODS

***Entamoeba* strains.** Trophozoites of *E. histolytica* strains (Table 1), *E. histolytica*-like Laredo, and *Entamoeba moshkovskii* were obtained from L. Diamond, National Institutes of Health, Bethesda, Md., and grown axenically in TYI-S-33 medium (8). *Entamoeba invadens* trophozoites were obtained from the American Type Culture Collection, Gaithersburg, Md., and cultured axenically. Clinical isolates of *E. histolytica* (Table 1) obtained from stools or liver abscess fluids submitted to the Microbiology Laboratory of the University of California, San Diego, Medical Center were cultured in Robinson medium (18) and subsequently transferred to TYSGM medium containing *Escherichia coli* 0111 (7). For zymodeme identification, parasites were lysed and electrophoresed on thin-layer starch gels, and the relative mobilities of glucose-phosphate isomerase, $NADP^+$ oxidoreductase, phosphoglucomutase, and hexokinase were determined (20). Other zymodeme-characterized strains (Table 1) were from P. Sargeaunt, London School of Hygiene and Tropical Medicine, London, England.

Preparation of *E. histolytica* nucleic acids. For DNA, trophozoites of *E. histolytica* growing in axenic cultures were washed in phosphate-buffered saline, lysed in 10 volumes of lysis buffer (1% *N*-lauroyl sarcosyl [sarcosyl], 8 M urea, 0.16 M sodium phosphate buffer [pH 6.8]), and extracted with phenol-chloroform. DNA in the lysis solution was bound to a hydroxylapatite column, which was washed in 8 M urea and 0.16 M phosphate and then in 0.19 M phosphate to remove carbohydrates and RNA, respectively. DNA was eluted with 0.48 M phosphate, dialyzed exhaustively in 10 mM Tris and 1 mM EDTA, pH 8, and concentrated by ethanol precipitation. RNA was prepared by lysing trophozoites in 5.7 M guanidinium isothiocyanate, 1% sarcosyl, and 5% β-mercaptoethanol and centrifuging them through a

TABLE 1. Strains of *Entamoeba* species and hybridization results

Species	Strain	Culture	Zymodeme[a]	Source	Hybridization with:[b]	
					pEH2	pEH6
E. histolytica	HM-1:IHSS	Axenic	II ((P)	Diamond	+	+
E. histolytica	CDC:0784:4	Axenic	II (P)	Diamond	+	+
E. histolytica	HK-9	Axenic	II (P)	Diamond	+	+
E. histolytica	200-NIH	Axenic	II (P)	Diamond	+	+
E. histolytica	53	Xenic	II (P)	Reed	+	+
E. histolytica	1519	Xenic	XIV (P)	Sargeaunt	+	+
E. histolytica	1453	Xenic	XIV (P)	Sargeaunt	+	+
E. histolytica	1704	Xenic	XIX (P)	Sargeaunt	+	+
E. histolytica	1721	Xenic	XIX (P)	Sargeaunt	+	+
E. histolytica	100	Xenic	I (NP)	Reed	+	+
E. histolytica	98	Xenic	III (NP)	Reed	+	+
E. histolytica	1749	Xenic	II (NP)	Sargeaunt	+	+
E. histolytica	Laredo	Axenic		Diamond	−	+
E. moshkovskii		Axenic		Diamond	−	+
E. invadens		Axenic		ATCC	−	+

[a] Zymodemes as defined by Sargeaunt et al. (20). P, Pathogenic; NP, nonpathogenic.
[b] Hybridization with pEH2, an *E. histolytica*-specific probe, or pEH6, an rRNA gene probe which hybridizes across *Entamoeba* species.

cesium chloride cushion (14). RNA was radiolabeled for Southern hybridization by a kinase reaction (14).

Screening of an *E. histolytica* genomic DNA library for highly repeated DNA sequences. Genomic DNA of the HM-1:IMSS strain of *E. histolytica* was partially cut with the restriction enzyme *Sau*3A to produce fragments ranging in molecular size from 300 to 5,000 base pairs (bp) on 1% agarose gels. The genomic DNA fragments were ligated into the plasmid pUC18, which was cut with *Bam*HI and treated with calf intestinal phosphatase, and were transformed into JM109 cells. Some 400 colonies were lifted onto nitrocellulose and hybridized with nick-translated (14) genomic DNA of *E. histolytica*.

Characterization of recombinant DNA clones. Twelve recombinant plasmid clones (pEH1 through pEH12) were selected, purified on cesium chloride gradients (14), radiolabeled, and hybridized to (i) dot blots of *Entamoeba* trophozoites (as prepared below), (ii) Southern blots of *E. histolytica* DNA which was restriction cut, separated on 1 or 2% agarose gels (1 μg per lane), and transferred to nitrocellulose or Genescreen Plus nylon membranes (Dupont, NEN Research Products, Boston, Mass.), or (iii) Northern (RNA) blots of *E. histolytica* RNA which was separated on denaturing gels containing 1% agarose and 6% formaldehyde or 1% agarose and 1.2% methyl mercury (14) and transferred to nitrocellulose. For nitrocellulose, hybridizations were performed at 42°C overnight in 50% formamide, 10× Denhardt solution, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and 500 μg of herring sperm DNA per ml (22), followed by three 30-min washes in 0.5% sodium dodecyl sulfate (SDS) and 0.1% SSC. For Genescreen Plus, hybridizations were performed at 42°C overnight in 50% formamide, 1 M NaCl, 1% SDS, 10% dextran, and herring sperm DNA, followed by two 30-min washes in 2× SSC at 22°C, 2× SSC and 1% SDS at 65°C, and 0.1× SSC at 22°C. The size of the insert DNA in recombinant DNA clones was determined by cutting with restriction enzymes and by agarose gel electrophoresis.

DNA sequence analysis. pEH5 and pEH11, two *E. histolytica*-specific recombinant DNA clones containing 145-bp inserts in the opposite orientation within the polylinker, and pEH3, pEH4, pEH6, and pEH10, four recombinant clones coding for rRNA gene sequences, were subcloned into M13mp18 and M13mp19. Single-stranded DNA was prepared from recombinant phage, and the DNA sequence was determined with the dideoxy chain termination method of Sanger et al. (19). Both pEH5 and pEH11 were sequenced completely in both directions, while partial sequences of the rRNA clones were compared with homologous sequences for the small-subunit rRNA sequence of *Dictyostelium discoideum* (15) to locate the clones on the rRNA gene. To determine whether there were any significant DNA sequence homologies between the 145-bp *E. histolytica*-specific diagnostic sequence and other known DNA sequences, the entire GenBank was searched by using a Lipman-Pearson FASTN algorithm with a k-tuple of 2.

Dot blots. *E. histolytica* DNA was diluted in 10 mM Tris and 1 mM EDTA, pH 8, denatured in 0.25 N NaOH, and spotted onto nylon membranes by using a 96-spot minifold apparatus (Schleicher & Schuell, Inc., Keene, N.H.). For determination of copy number, inserts of pEH5 and pEH6 were electroeluted from agarose gels, diluted, and spotted onto nylon membranes and hybridized in parallel with trophozoites. Trophozoites of axenized amoebae were washed in phosphate-buffered saline, counted with a hemacytometer, diluted, lysed in 10 mM Tris, 100 mM EDTA, 0.1% Triton X-100, and 100 μg of proteinase K per ml for 60 min at 50°C (3), denatured with NaOH, and spotted. Xenic cultures of trophozoites were separated from most bacteria by means of a Percoll gradient (17), extensively washed in phosphate-buffered saline, frozen in 10 mM Tris and 100 mM EDTA, and shipped from San Diego to Boston. Frozen xenic cultures were thawed, diluted, and lysed before application to nylon membranes.

Determination of the best conditions for detecting *E. histolytica* parasites in stool. Optimal conditions for preparing parasite DNA from stool samples were determined by trial and error experiments in which cultured *E. histolytica* trophozoites were mixed with uninfected stools, dot blotted, and hybridized with the *E. histolytica*-specific clone pEH12. Samples containing 1,000 to 5,000 *E. histolytica* trophozoites and 200 μl of stool were diluted into 800 μl of 10 mM Tris buffer and then incubated for 1 h at 37°C with either 0 to 500 mM EDTA, 0 to 1% SDS, or 0.5 N NaOH. Subsequently, stool bacteria and debris were removed by centrifugation for 1 min in a microcentrifuge. Then, 100 μl of each supernatant was spotted in duplicate onto nylon membranes, denatured with 0.5 N NaOH if this had not been done already, and

hybridized with radiolabeled pEH12. Alternatively, the 1,400-bp insert of pEH12 was cut out of the plasmid, separated on an agarose gel, and radiolabeled to reduce background hybridization.

Because it is not possible to make *E. histolytica* cysts in vitro, *E. invadens* cysts prepared by removing glucose from the medium and incubating for 4 days (25) were used to determine the best way to release DNA from cysts. Contaminating *E. invadens* trophozoites were removed from the cyst preparations by lysis in 1% Nonidet P-40, which did not disrupt the cysts. *E. invadens* cysts (1,000 to 5,000/ml) were washed three times by centrifugation in phosphate-buffered saline and then incubated for 1 h at 37°C either in 1% SDS, 5.7 M guanidinium isothiocyanate, and 0.5 N NaOH in 0.14 M NaCl or in 100 ng of proteinase K per ml. Alternatively, cysts were frozen in dry ice and ethanol and thawed in a 37°C water bath three times, boiled for 5 min in 10% SDS, or sonicated for 1 min with a probe sonicator. Cyst walls were pelleted with a microcentrifuge, and samples of the supernatants were spotted onto nylon filters and hybridized with radiolabeled genomic *E. invadens* DNA, because the *E. histolytica*-specific probes did not bind to them.

Preparation of patient stool samples. Stool samples (123) came from patients referred by their physicians to the Laboratorio de Parasitologia in Mexico City for identification of intestinal parasites. Cysts and eggs were concentrated by flotation from 10 to 50 g of stool (5), and parasites were identified by morphology by microscopic examination. Parallel samples contained 1 g of untreated stool directly frozen or cysts concentrated by flotation. The concentrated cysts were divided into two samples: one frozen directly and the other fixed with 10% Formalin before freezing.

To each patient sample, 0.5 ml of 10 mM Tris and 500 mM EDTA, pH 8, were added upon thawing to prevent degradation of DNA, and samples were frozen in ethanol-dry ice and thawed in water at 37°C for three cycles to rupture cysts. One hundred microliters of each sample was directly applied to the minifold after a spin (14,000 rpm, 1 min) to remove unlysed bacteria and stool debris, and the DNA on the membranes was subsequently denatured with 0.5 N NaOH and 1.5 M NaCl. To prevent background hybridization of vector sequences to stool, the 1,400-bp insert of pEH12, a recombinant clone specific for *E. histolytica* DNA, was cut out with *Eco*RI and *Hin*dIII, electrophoresed in low-melting-point agarose, and radiolabeled by random-oligomer priming (10). After all the DNA hybridizations of the stool samples were completed and positive and negative samples were identified, the microscopic diagnoses were sent from Mexico City. The sensitivity of the hybridization probe was calculated by dividing the number of samples identified as positive with both microscopy and DNA hybridization by the number positive with microscopy alone. The specificity of the probe was calculated by dividing the number of samples identified as negative with both microscopy and the DNA probe by the number negative with microscopy alone.

RESULTS

Specificity of the DNA hybridization probe for *E. histolytica*. When a genomic library of *E. histolytica* was screened for highly repeated DNA sequences, two classes of recombinant plasmid clones were obtained. In the first class were four clones (pEH2, pEH5, pEH11, and pEH12) which appeared specific for *E. histolytica* DNA sequences. These clones bound to all the axenized strains of *E. histolytica* tested but did not bind to closely related *E. histolytica*-like Laredo, *E. moshkovskii*, or *E. invadens* parasites (Fig. 1A and Table 1). The clones did not bind to human, *Giardia*, or bacterial DNA (data not shown). These *E. histolytica*-specific clones recognized all *E. histolytica* trophozoites tested, including three pathogenic (II, XIV, and XIX) and two nonpathogenic (I and III) zymodemes of *E. histolytica* growing in xenic cultures (Fig. 2 and Table 1).

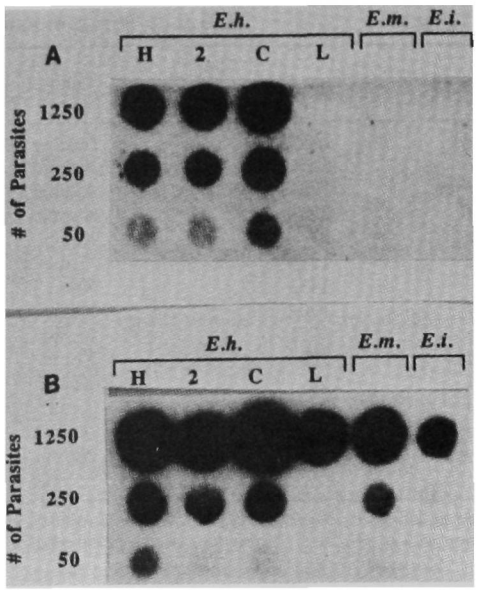

FIG. 1. Specificity of binding of recombinant DNA clones to dot blotted *Entamoeba* species. (A) Nick-translated pEH2, one of four diagnostic hybridization probes, bound to trophozoites of HM-1:IHSS (lane H), 200-NIH (lane 2), and CDC:0784:4 (lane C) strains of *E. histolytica* (*E.h*) but did not bind to trophozoites of *E. histolytica*-like Laredo (lane L), *E. moshkovskii* (*E.m.*), or *E. invadens* (*E.i.*). (B) In contrast, pEH6, a cloned portion of the small-subunit rRNA gene of *E. histolytica*, bound to all *Entamoeba* species tested.

In contrast, a second class of recombinant plasmid clones (pEH3, pEH4, pEH6, and pEH10) recognized repeated DNA sequences which were conserved across *Entamoeba* species (Fig. 1B and Table 1). These nonspecific clones appeared to code for portions of the rRNA gene of *E.*

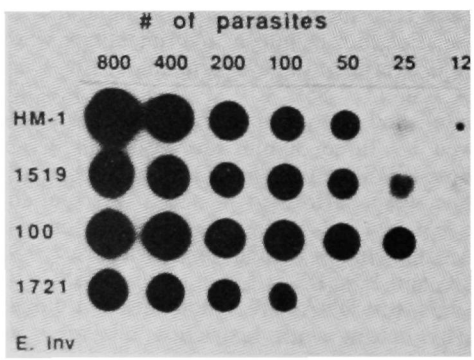

FIG. 2. Representative hybridization of the diagnostic recombinant DNA clone pEH2 to zymodeme-characterized *Entamoeba* species. Radiolabeled pEH2 insert bound to axenically cultured HM-1:IHSS (HM-1) and xenic cultures of isolates 1519, 100, and 1721 (Table 1) with similar affinities, suggesting about the same target copy number in each. As a control, pEH2 did not bind to *E. invadens* (E. inv) spotted in parallel.

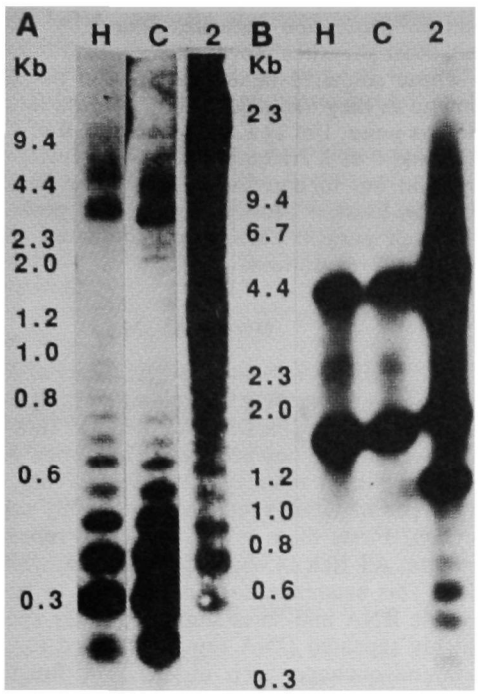

FIG. 3. Southern blots of *E. histolytica* DNA hybridized with the species-specific clone pEH5. DNAs of HM-1:IHSS (lane H), CDC: 0784:4 (lane C), and 200-NIH (lane 2) were cut with *Taq*I (A) or *Sau*3A (B). The ladder produced by incomplete cutting with *Taq*I shows that the sequence recognized is a tandem repeat. With *Sau*3A, there was a 1.2-kilobase (Kb)-long restriction fragment length polymorphism, which distinguished 200-NIH amoebae from the other two *E. histolytica* strains.

histolytica, because they hybridized to Northern blots of *E. histolytica* RNA and to bands on Southern blots of *E. histolytica* DNA, which were also recognized by radiolabeled *E. histolytica* RNA (data not shown; 4). Partial DNA sequencing of the clone pEH6 showed homology with the small-subunit rRNA gene of *D. discoideum* (16) beginning at about 300 bp from the 5′ end and continuing to the 3′ end of the gene (data not shown).

Molecular analysis of the *E. histolytica*-specific recombinant clones. The four *E. histolytica*-specific recombinant DNA clones showed nearly identical patterns on Southern blots of restriction-enzyme-cut *E. histolytica* DNA and so recognized the same highly repeated sequences within the parasite genome (Fig. 3). The sequences recognized were tandem 145-bp long repeats, as shown by the ladders formed when the genomic DNA was partially digested with *Taq*I (Fig. 3A). A degeneracy in the repeat or a modification of a subset of the repeat sequences was suggested by the failure to cut completely with *Sau*3A (Fig. 3B).

When parasite DNA was cut with *Sau*3A and hybridized with the diagnostic clone, there was a 1.2-kilobase band in the DNA from the 200-NIH strain that was not present in the DNA of the HM-1:IHSS or CDC:0784:4 strain (Fig. 3B). This restriction fragment length polymorphism may have potential value in developing a method to distinguish strains of *E. histolytica*, as has been shown for restriction fragment length polymorphisms defined by an rRNA gene probe (4).

pEH5 and pEH11 each contained single 145-bp inserts, which were in opposite directions within the polylinker of the plasmids. The DNA sequences of these inserts were identical, AT-rich, and contained no significant open reading

5′ GAATTATTCA AAATGGTCGT CGTCTAGGCC 30
AAAATATTTT TTGACCAATT TACACCGTTG 60
ATTTTCGATT TCCTAAGAAC CTCACCATTT 90
TTAATGAAAA GTACTAAATA CAAAGTACAA 120
TAATTTCTAA CTGGGAAAAT CGATC 3′ 145

FIG. 4. DNA sequence of 145-bp tandemly repeated *E. histolytica*-specific sequence. The DNA sequences of the inserts of pEH5 and pEH11 were identical. Analysis of the sequence revealed no significant open reading frames.

frames (Fig. 4). There were no DNA sequences found in a search of the entire GenBank with significant homology to the 145-bp *E. histolytica* diagnostic insert. pEH2 and pEH12 contained 1,800- and 1,400-bp inserts, which were composed of tandem repeats of 145 bp as determined by partial digests with *Taq*I of gel-purified inserts (data not shown). The *E. histolytica*-specific recombinant clones did not bind to Northern blots of parasite RNA, suggesting that the repeated DNA sequences were not transcribed or that mRNAs were too few or too unstable to be detected.

Sensitivity of the diagnostic clones. The *E. histolytica*-specific recombinant clones were very sensitive, detecting as few as eight cultured *E. histolytica* trophozoites in dot blots after an overnight exposure (Fig. 5A). Using dilutions of the 145-bp insert spotted in parallel to measure amoeba DNA content, we estimated that each parasite contains 50 to 100 fg of the 145-bp repeat (Fig. 5A). If we assume that each cell

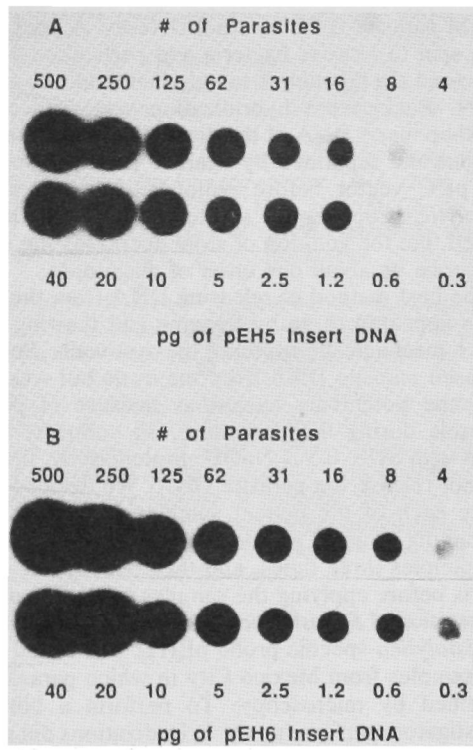

FIG. 5. Estimation of copy number of inserts of pEH5 and pEH6 in HM-1:IHSS trophozoites. Cultured parasites were diluted and spotted in parallel with dilutions of purified inserts of pEH5, which contains a 145-bp species-specific DNA sequence, and pEH6, which contains 1,600 bp of the small-subunit rRNA gene of *E. histolytica*. Radiolabeled pEH5 (A) and pEH6 (B) inserts both detected as few as eight parasites, and there appeared to be about 50 to 100 fg of each target DNA per parasite.

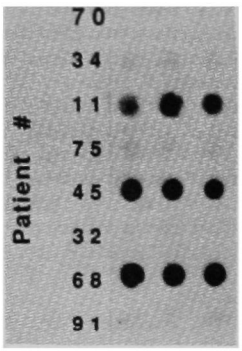

FIG. 6. Representative hybridization of the diagnostic DNA probe with stool samples. Clinical samples were frozen and thawed, centrifuged, spotted in triplicate, and hybridized with a radiolabeled pEH12 insert. On the left is the number of each stool sample. Numbers 11, 45, and 68 are positive by our criterion.

contains about 500 fg of DNA (9), the 145-bp repeat comprises at least 10% of the amoeba DNA. There appeared to be 50 to 100 fg of the pEH6 insert DNA per trophozoite (Fig. 5B), suggesting that the rRNA genes may also compose 10% of the parasite genome.

Conditions for detecting *E. histolytica* parasites in stool. Cultured *E. histolytica* trophozoites were mixed with uninfected stools, dot blotted, and probed with the diagnostic clone pEH12 to determine by trial and error conditions for preserving target DNA and eliminating background hybridization. We found that the addition of 500 mM EDTA to stool samples was necessary to prevent the rapid degradation of parasite DNA by stool DNases. A brief microcentrifuge spin to remove bacteria and particulate debris greatly increased the amount of sample that could be applied to the filters. Background hybridization was reduced when the 1,400-bp insert DNA of the diagnostic sequence pEH12 was cut out and separated by agarose gel electrophoresis from the pUC vector before radiolabeling. Comparison of *E. histolytica* trophozoites spotted with or without stool suggested that the addition of stool decreases the sensitivity of the probe by about one order of magnitude.

The best method of releasing DNA from the *E. invadens* cysts appeared to be by freezing and thawing three times, which mechanically ruptured the cyst walls. Sonication also released parasite DNA from the cysts but was labor intensive and potentially hazardous because of production of aerosols during the procedure. In contrast, treatment of cysts with SDS, 0.5 N NaOH, proteinase K, or guanidinium did not release the parasite DNA. We decided therefore to dilute each of the patient samples in four parts of buffer containing 10 mM Tris and 500 mM EDTA, freeze and thaw the samples three times, and then pellet away bacteria and debris before applying the samples to the nylon filters.

Detection of *E. histolytica* parasites in clinical samples. The *E. histolytica*-specific probe pEH12 was tested against clinical samples from Mexico City in which parasites had been identified by microscopy. To perform a blind trial, the investigators performing the hybridizations did not know the results of the microscopy until after the hybridizations were performed and scored. Stool or purified cysts and ova were frozen and thawed to break open cysts in the presence of EDTA to prevent DNase activity, dot blotted onto nylon filters, and hybridized with radiolabeled insert DNA of pEH12 (Fig. 6). The DNA hybridization probe correctly identified 25 of 25 stools containing *E. histolytica* parasites, for a sensitivity of 1.0. The probe found seven stools to contain *E. histolytica* parasites found by microscopy to contain other parasites, not *E. histolytica*, for a specificity of 0.93. These so-called false-positives did not appear to be systematic as they included 4 of 36 *Giardia lamblia*, 2 of 20 *Endolimax nana*, 1 of 14 *Entamoeba coli*, 0 of 8 *Chilomastix mesnili*, and 0 of 3 *Trichuris trichiuria*. Ninety-one samples were found not to contain *E. histolytica* parasites by the DNA hybridization probe. Finally, the probe recognized equally well parasites from unfractionated stool or from purified cyst preparations.

DISCUSSION

DNA hybridization probes for diagnosis of *E. histolytica* were made by identifying and cloning highly repeated and species-specific amoeba DNA sequences. In a blind study, these diagnostic probes accurately identified *E. histolytica* parasites in patient samples from Mexico City.

Two types of DNA sequences are highly repeated in *E. histolytica*. First, there is the tandemly repeated, slightly degenerate, AT-rich 145-bp long sequence, which is species specific. This sequence does not appear to be transcribed into stable RNA and so is similar to other species-specific and highly repeated DNA sequences used for diagnosis of malaria and onchocerciasis (3, 21). The function of these parasite sequences and similar species-specific repeated DNA sequences in the mammalian genome is not known (13). Second, the rRNA genes of *E. histolytica* are also highly repeated, presumably to amplify the amount of rRNA produced by the parasites. Because major portions of the rRNA genes are conserved across species (15), the complete rRNA genes were not useful as diagnostic probes. However, portions of the rRNA genes that appear to be species specific have recently been identified (4).

The DNA hybridization probe developed here recognized all *E. histolytica* parasites tested but did not recognize *E. histolytica*-like Laredo parasites. These Laredo parasites likely represent a different species from *E. histolytica* because they are nonpathogenic, grow at 25 instead of 37°C, do not bind monoclonal antibodies which bind to *E. histolytica* (6), and fail to hybridize with portions of the *E. histolytica* rRNA gene (4).

The DNA hybridization probes did not distinguish between *E. histolytica* zymodemes. The potential importance of zymodeme analysis is that it may distinguish potentially harmful from harmless parasites (20). A recent report suggests that pathogenic and nonpathogenic zymodemes may be distinguished by monoclonal antibody binding to cultured trophozoites (23). However, whether the zymodeme of a parasite is fixed by its genotype or is a phenotypic trait with differential expression dependent upon the environment of the parasite is presently a subject of controversy (16).

When compared with microscopy of cysts isolated from stool by flotation, which is the gold standard for clinical diagnosis of *E. histolytica*, the DNA hybridization probe performed well. The probe correctly identified 25 of 25 specimens containing *E. histolytica* and called positive another 7 of 98 specimens called negative by microscopy. The latter positives likely represent errors of the probe but may represent errors of the microscopist (samples were not available for review by microscopy). Fortunately, these errors did not appear to be systematic in that other common stool parasites such as *G. lamblia*, *E. nana*, *T. trichiuria*, *C. mesnili*, or *E. coli* were not overrepresented. Similar false-positives have been reported for an ELISA that uses a

monoclonal antibody to detect *E. histolytica* directly from stool (6). The ELISA correctly identified all samples containing *E. histolytica* parasites seen by microscopy but showed an apparent false-positive rate of 13 of 46 positives (6). Previous ELISAs detected trophozoites but not cysts in stool (2, 11).

The advantage of either the DNA hybridization probe or the ELISA over microscopy is the brevity of specimen preparation: stool is directly spotted onto nylon filters or is solubilized and placed in microdilution wells, respectively. In addition, with the DNA probe as many as 96 specimens per sheet times 10 to 20 sheets can be hybridized in parallel. Because DNA hybridization is a simple and potentially inexpensive method for diagnosing *E. histolytica*, this technique might be used to survey large numbers of persons to estimate accurately the prevalence of *E. histolytica* infection and to evaluate the effects of control measures to limit infection with amoebae. Finally, methods to use the polymerase chain reaction to amplify target DNA and increase the sensitivity of the amoeba probe and to use nonradioactive detection systems to replace ^{32}P-labeled probes are presently being developed in our lab.

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LITERATURE CITED

1. **Allason-Jones, E., A. Midel, P. Sargeaunt, and P. Williams.** 1986. *Entamoeba histolytica* as a commensal intestinal parasite in homosexual men. N. Engl. J. Med. **315**:353–356.
2. **Anand, P., B. Malaviya, P. Das, M. A. Mateen, C. M. Habibullah, and S. R. Das.** 1985. Multilayer-enzyme linked immunoabsorbent assay (MI-ELISA) for detection of *Entamoeba histolytica* trophozoite coproantigen. Immunol. Invest. **14**:443–453.
3. **Barker, R. H., Jr., H. Suebsaeng, W. Rooney, G. C. Alecrim, H. V. Dourado, and D. F. Wirth.** 1986. Specific DNA probe for diagnosis of *Plasmodium falciparum* malaria. Science **231**: 434–436.
4. **Bhattacharya, S., A. Bhattacharya, and L. S. Diamond.** 1988. Comparison of repeated DNA from strains of *Entamoeba histolytica* and other *Entamoeba*. Mol. Biochem. Parasitol. **27**: 257–262.
5. **Biagi, F., and J. Portilla.** 1957. Comparison of methods of examining stools for parasites. Am. J. Trop. Med. Hyg. **6**: 906–911.
6. **del Moro, R., A. Oliva, P. Herion, R. Capin, and L. Ortiz-Ortiz.** 1987. Diagnosis of *Entamoeba histolytica* in feces by ELISA. J. Clin. Lab. Anal. **1**:322–325.
7. **Diamond, L. S.** 1982. A new liquid medium for xenic culture of *Entamoeba histolytica*. J. Parasitol. **68**:958–959.
8. **Diamond, L. S., D. R. Harlow, and C. C. Cunnick.** 1978. A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. Trans. R. Soc. Trop. Med. Hyg. **73**:431–432.
9. **Edman, U., I. Meza, and N. Agabian.** 1987. Genomic and cDNA actin sequences from a virulent strain of *Entamoeba histolytica*. Proc. Natl. Acad. Sci. USA **84**:3024–3028.
10. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
11. **Grundy, M. S.** 1982. Preliminary observations using a multilayer ELISA method for the detection of *Entamoeba histolytica* trophozoite antigens in stool samples. Trans. R. Soc. Trop. Med. Hyg. **76**:396–400.
12. **Guerrant, R. L.** 1986. Amebiasis: introduction, current status, and research questions. Rev. Infect. Dis. **8**:218–227.
13. **Lewin, B.** 1985. Genes, 2nd ed., p. 381–392. John Wiley & Sons, Inc., New York.
14. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **McCarroll, R., G. J. Olsen, Y. D. Stahl, C. R. Woese, and M. L. Sogin.** 1983. Nucleotide sequence of *Dictyostelium discoideum* small-subunit ribosomal ribonucleic acid inferred from the gene sequence: evolutionary implications. Biochemistry **22**:5858–5868.
16. **Mirelman, D., R. Bracha, A. Chayen, A. Aust-Kettis, and L. S. Diamond.** 1986. *Entamoeba histolytica*: effect of growth conditions and bacterial associates on isoenzyme patterns and virulence. Exp. Parasitol. **62**:142–148.
17. **Reed, S. L., J. G. Curd, I. Gigli, F. D. Gillin, and A. I. Braude.** 1986. Activation of complement by pathogenic and nonpathogenic *Entamoeba histolytica*. J. Immunol. **136**:2265–2270.
18. **Robinson, G.** 1968. The laboratory diagnosis of human parasitic amoebae. Trans. R. Soc. Trop. Med. Hyg. **62**:285–294.
19. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
20. **Sargeaunt, P. G., J. E. Williams, and J. D. Greene.** 1978. The differentiation of invasive and non-invasive *E. histolytica* by isoenzyme electrophoresis. Trans. R. Soc. Trop. Med. Hyg. **72**:519–521.
21. **Shah, J. S., M. Karam, W. F. Piessens, and D. F. Wirth.** 1987. Characterization of an *Onchocerca*-specific DNA clone from *Onchocerca volvulus*. Am. J. Trop. Med. Hyg. **37**:376–384.
22. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
23. **Strachan, W. D., W. D. Spice, P. L. Chiodini, A. H. Moody, and J. P. Ackers.** 1988. Immunological differentiation of pathogenic and non-pathogenic isolates of *Entamoeba histolytica*. Lancet **i**:561–563.
24. **Trissl, D.** 1982. Immunology of *Entamoeba histolytica* in human and animal hosts. Rev. Infect. Dis. **4**:1154–1184.
25. **Vazquezdelara-Cisneros, L. G., and A. Arroyo-Begovich.** 1984. Induction of encystation of *Entamoeba invadens* by removal of glucose from the culture medium. J. Parasitol. **70**:629–633.
26. **Walsh, J. A.** 1986. Problems in recognition and diagnosis of amebiasis: estimation of the global magnitude of morbidity and mortality. Rev. Infect. Dis. **8**:228–238.

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Diagnosis of *Giardia lamblia* Infections by Detection of Parasite-Specific Antigens

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Antigen detection methods may facilitate diagnosis of *Giardia lamblia* in stool specimens. As determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis and immunoblotting, *G. lamblia* cysts and trophozoites share several antigens, especially in the 65-kilodalton and 30- to 34-kilodalton regions. By using blind methods, we compared results obtained by counterimmunoelectrophoresis using cyst-immune rabbit serum and by enzyme-linked immunosorbent assay (ELISA) using trophozoite-immune rabbit serum with results obtained by microscopic examination of a preserved, concentrated, and permanently stained stool specimen. Results were similar when these three methods were used to examine 118 stool specimens from clinical microbiology laboratories (53 specimens with *G. lamblia*) and specimens from 239 day-care-center toddlers (39 specimens with *G. lamblia*). Compared with microscopy, we found, for counterimmunoelectrophoresis and ELISA, respectively: sensitivity, 88 versus 94%; specificity, 97 versus 95%; positive predictive value, 86 versus 76%; negative predictive value, 98 versus 97%; and concordance, 89%. The false-positive rate by ELISA was 24% (10 of 42) in day-care-center toddlers but only 3% (1 of 32) in healthy adults ($P < 0.04$) as corroborated by microscopy. This discrepancy suggests that the ELISA may be more sensitive than microscopy, which is considered the reference standard, and that results may be dependent, in part, on the epidemiology of the infection in the study subjects.

Reliable information on the epidemiology and natural history of *Giardia lamblia* infections has been limited because the sensitivity of microscopic diagnosis of the parasite in concentrated stool specimens varies from 46 to 95% (1, 6, 12, 13). Examination of multiple samples may be required for diagnosis, particularly from persons with early (9) or chronic infections (3). Other diagnostic methods, some of which require sampling of the small bowel, are more expensive, uncomfortable, and invasive (6, 10, 13). Stool antigen detection systems for the diagnosis of *G. lamblia* infections have been reported to be highly sensitive and specific (2, 7, 14, 23, 24). These methods may permit large numbers of stool samples to be tested rapidly and may reduce technician time and bias among observers.

Standard diagnostic methods are designed to detect the specific stages of the life cycle of *G. lamblia*, cysts and trophozoites. Microscopic examination of stool samples most often detects cysts (4, 21), whereas duodenal aspiration and biopsy primarily identify trophozoites (6). In contrast, antigen detection systems, although also designed to detect the distinct stages of the life cycle, show similar diagnostic accuracies. We identified the antigens detected by two antigen detection methods to understand the relationship between cyst and trophozoite antigens and to better define and standardize the assay systems. We performed the first blinded comparison of three noninvasive methods for diagnosis of *G. lamblia* infections from stool samples: microscopic examination, detection of cyst antigens by counterimmunoelectrophoresis (CIE), and detection of trophozoite antigens by enzyme-linked immunosorbent assay (ELISA).

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MATERIALS AND METHODS

Antigen preparation. *G. lamblia* Portland-1 trophozoites, graciously provided by Ernest Meyer, were grown and prepared as previously described (8). Purified cysts were prepared from human fecal specimens, as previously described (2), and were kindly provided, in part, by Charles Hibler. The cyst purity was assessed by microscopy (2), and the preparations were sonicated, centrifuged at 1,000 × *g* for 30 min, and preserved at −70°C.

Antigen analysis. Rabbit sera used in the antigen detection systems were compared by ELISA as previously described (8), except that the solid-phase antigens were whole frozen *G. lamblia* Portland-1 trophozoites (10^4 organisms per well) or purified *G. lamblia* cysts (10^3 cysts per well). The endpoint was defined as the highest dilution of rabbit serum that resulted in an optical density (OD) greater than 0.200 at 405 nm. The specificities of the antibodies detected were determined by adsorption of immune serum four times for 45 min each time at 37°C on a circular rotator with each of the following Formalin-fixed organisms (number of organisms per adsorption): *G. lamblia* Portland-1 trophozoites (6×10^6); *Trichomonas vaginalis* cultured from a symptomatic patient (6×10^6); *Cryptosporidium* oocysts and freshly excysted sporozoites (10^7), provided by Charles Sterling and

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Michael Arrowood; *Campylobacter jejuni* (10^9); enterotoxigenic *Escherichia coli* (10^9); and *Candida albicans* (10^9).

Whole-cell preparations of purified *G. lamblia* cysts and trophozoites were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described (8). Immunoblots of trophozoite antigens were prepared with serum from New Zealand White rabbits immunized with *G. lamblia* cysts or trophozoites and used in the CIE and ELISA systems, respectively (2, 8).

Stool specimens. In Denver, Colo., in 1985, we obtained (i) single unpreserved stool samples from each of 239 randomly selected toddlers in day-care centers (T. Novotny, R. S. Hopkins, P. Shillam, and E. N. Janoff, Abstr. Annu. Conf. Epidemic Intelligence Service, Centers for Disease Control, 1986) and (ii) 118 samples submitted to the clinical microbiology laboratories of the University of Colorado, Veterans Administration Medical Center, and Denver General Hospital from 86 persons with diarrhea and 32 healthy adults with no history of gastrointestinal symptoms or known *G. lamblia* exposure within the past 2 months. Fresh stool specimens were preserved in 10% Formalin or in polyvinyl alcohol, and a third fraction was stored within 12 h of collection from test subjects without preservative at −70°C until processed.

Microscopy. The entire 22-mm^2 cover slip from each Formalin-ether (or Formalin-ethyl acetate) concentrate was examined at a magnification of × 10, as was each trichrome stain of polyvinyl alcohol-preserved stool specimen examined under an oil immersion lens. Each concentrated specimen was stained for *Cryptosporidium* oocysts and sporozoites with the modified Kinyoun acid-fast (cold) technique (11), and the entire slide was scanned for 5 min at a magnification of × 40. Positive specimens were confirmed by examination under an oil immersion lens. Identification of *G. lamblia* by microscopic examination of preserved concentrate or of a permanently stained smear was considered the reference standard for the antigen detection systems. The numbers of *G. lamblia* organisms detected were graded as rare to few, moderate, or many in 31 of 39 positive specimens from day-care-center toddlers.

Antigen detection. The CIE assay was performed as previously described (2), with serum from cyst-immune rabbits. For the ELISA system, New Zealand White rabbits were immunized with axenically grown *G. lamblia* Portland-1 trophozoites. A crude serum immunoglobulin fraction was precipitated with ammonium sulfate and dialyzed, and a portion was conjugated to horseradish peroxidase (22). Wells of microdilution plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with unlabeled rabbit anti-*G. lamblia* antibody in 100 mM carbonate buffer (pH 9.0) overnight at 4°C. Freshly thawed fecal specimens were diluted 1:2 with 50% fetal calf serum–0.01 M phosphate-buffered saline (pH 7.2) plus 2 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co., St. Louis, Mo.) and 0.1% thimerosal, vortexed, and allowed to settle for 30 min. The supernatant was incubated for 1 h at 37°C in duplicate wells. After wells were washed three times by using a multichannel pipette, peroxidase-conjugated rabbit anti-*G. lamblia* antibody was applied, incubated for 1 h at 37°C, and developed with *o*-phenylenediamine in 53 mM citrate–94 mM phosphate buffer (pH 5.0) for 15 min. The reaction was stopped with 2.5 N H_2SO_4, and the A_{490} was measured on a microELISA plate reader (MR580; Dynatech). Positive and negative controls were included on each plate. Specimens which produced an OD of less than 0.100 were considered negative, those with an OD between 0.100 and 0.250 were considered equivocal, and those which produced an OD above 0.250 were considered positive on the basis of results previously obtained from specimens characterized by microscopy (C. V. Knisley, P. G. Englekirk, L. K. Pickering, S. West, and E. N. Janoff, Am. J. Clin. Pathol., in press). Preliminary studies showed that an OD of 0.250 resulted from the addition of 250 trophozoites (1 ng of protein) to a test well. We decided in advance to consider any specimens which produced equivocal results upon CIE or ELISA to be negative for the purpose of analysis.

We evaluated each method on the basis of the number of specimens tested by that method; all 357 specimens were tested by microscopic examination, 327 (92%) were tested by CIE, and 312 (87%) were tested by ELISA. We compared the three assays by using results from the 282 (79%) specimens tested by all three methods. To investigate the reproducibility of test results, nine stool samples were tested in a blind manner in duplicate by CIE, as were six by ELISA. ODs by ELISA were compared for 20 samples tested twice on separate days to establish the test-retest reliability.

Statistics. Results were evaluated by the chi-square and Fisher exact tests as indicated.

RESULTS

Antigen analysis. The trophozoite-immune rabbit serum used in the ELISA system showed a titer of 1:25,600 with the trophozoites and a titer of 1:400 with the cysts as the solid-phase capture antigens. The cyst-immune rabbit serum used in the CIE system showed a titer of 1:200 with the trophozoites and a titer of 1:3,200 with the cysts as the solid-phase capture antigens. Preimmune rabbit serum showed a titer of <1:100, the lowest dilution tested, with both antigens. Trophozoite-immune serum showed the greatest decrease in titer following adsorption with *G. lamblia* trophozoites, from 1:25,600 to 1:3,200. Titers after adsorption with other protozoal, bacterial, and fungal organisms remained 1:12,800.

Eight distinct bands, with major bands at 88, 60, 55, 48, 33, and 31 kilodaltons (kDa), were resolved from the soluble *G. lamblia* cyst preparation (Fig. 1). Silver staining of solubilized trophozoites also produced bands of 189, 88, 75, 68, 55, 47, and 34 kDa (13). Immunoblotting of whole trophozoites with cyst-immune and trophozoite-immune rabbit sera revealed common trophozoite antigens of 185, 65, and 57 kDa and produced the strongest reactivity with heavy bands of 33 and 30 kDa (Fig. 2).

Antigen detection in stool samples. The sensitivities and specificities of the two antigen detection methods (CIE and ELISA) were similar to results obtained by microscopy with stool specimens from day-care-center toddlers and with specimens submitted to clinical laboratories (Table 1). The numbers of organisms identified by microscopy in 31 of 39 day-care-center samples examined in which *G. lamblia* was identified were rare to few in 48%, moderate in 16%, and many in 35%. Both CIE and ELISA showed negative results for two specimens in which *G. lamblia* was detected by microscopy. Two stool samples taken from patients 2 weeks after they started therapy for documented *G. lamblia* infections were negative by microscopy; one sample was positive by CIE, as was the one sample tested by ELISA. Thirteen stool samples with other parasites, including *Entamoeba histolytica* (n = 2), *Cryptosporidium* spp. (n = 7), *Blastocystis hominis* (n = 3), and others (*Entamoeba coli*, *Entamoeba nana*, *Trichuris trichuris*) showed negative results by both methods. Nine samples tested in a blind manner in duplicate by CIE gave concordant results (seven positive and two

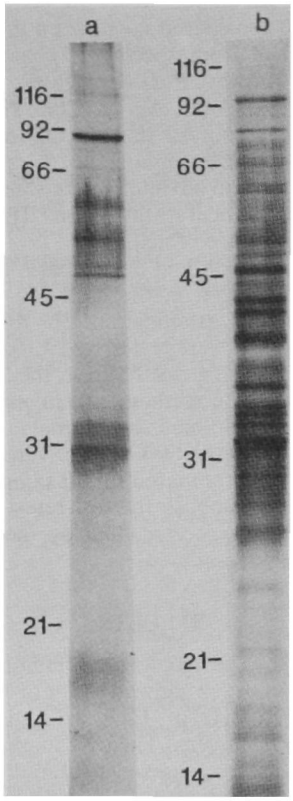

FIG. 1. Silver-stained 12% polyacrylamide SDS-PAGE preparations of solubilized *G. lamblia*. Lanes: a, cysts from an infected patient; b, axenically grown Portland-1 strain trophozoites.

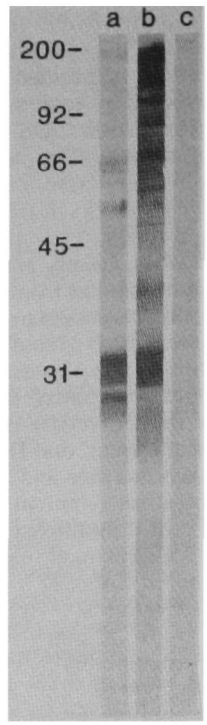

FIG. 2. Immunoblots of *G. lamblia* Portland-1 trophozoites resolved on 12% polyacrylamide SDS-PAGE gels. Nitrocellulose strips were reacted with serum from rabbits immunized with *G. lamblia* cysts (lane a), *G. lamblia* trophozoites (lane b), and preimmune serum (lane c).

negative), as did five of six samples tested by ELISA (three of four positive and two negative). The test-retest reliability of ODs produced with 20 samples tested on two occasions by ELISA was high (correlation coefficient = 0.953), and the mean values did not differ by the paired *t* test. The presence or absence of symptoms, such as diarrhea and abdominal pain, in the patients from whom the specimens were obtained did not affect the sensitivities or specificities of the two methods (data not shown). The concordance rates between the two antigen detection methods was 92% (196 of 214) for samples without *G. lamblia* and 81% (55 of 68) for samples with *G. lamblia* as corroborated by microscopy.

The predictive value of a positive result was lower in samples from day-care-center toddlers than from routine samples from clinical microbiology laboratories. When microscopy results were considered the reference standard, false-positive rates were higher among day-care-center toddlers, among whom rates of exposure to and carriage of *G. lamblia* are often higher (1, 16; Novotny et. al., Abstr. Annu. Conf. Epidemic Intelligence Service, 1986) than among healthy adults with no known *G. lamblia* exposure, who are less likely to be infected (Table 2).

Of the samples tested by CIE, 5% (16 of 327) showed equivocal results (grey zones); 13% of those tested by ELISA (41 of 312) showed equivocal results. As part of our protocol prior to testing, any equivocal result was defined as a negative test result for the purposes of analysis. In the clinical setting, these results would have been called equivocal and a second specimen would have been requested. No *G. lamblia* was identified by microscopy in 81% (13 of 16)

TABLE 1. *Comparison of antigen detection methods with microscopy for diagnosis of G. lamblia infections*

Source of specimens (diagnostic test)[a]	No. tested	No. (%) with *G. lamblia*	Sensitivity (%)[b]	Specificity (%)[b]	Positive predictive value (%)	Negative predictive value (%)
Day-care-center toddlers						
MICRO	239	39 (16)	100	100		
CIE	212	35 (17)	88	97	86	98
ELISA	218	42 (19)	94	95	76	99
Clinical microbiology laboratory						
MICRO	118	53 (45)	100	100		
CIE	115	48 (42)	96	97	96	93
ELISA	94	42 (45)	90	91	88	92

[a] MICRO, Microscopic examination of a single preserved, concentrated, and permanently stained specimen; CIE, CIE assay for cyst antigen; ELISA, ELISA for trophozoite antigen.

[b] Microscopic examination is considered the reference standard for this analysis.

TABLE 2. Comparison of false-positive rates using antigen detection methods in two epidemiologically distinct groups

Group	No. false-positive/total no. (%) by[a]:	
	CIE	ELISA
Day-care-center toddlers	5/35 (14)	10/42 (24)
Healthy adults	0/32[b] (0)	1/32[b] (3)

[a] For the purposes of this analysis, results are considered as false-positives if the sample produced a positive result by ELISA or CIE but no organisms were identified by microscopic examination of a single concentrated and permanently stained sample from each subject.
[b] $P < 0.05$, Fisher exact test.

and 90% (37 of 41) of these samples, which were equivocal by CIE and ELISA, respectively. Exclusion of these samples from the analysis did not significantly change the evaluation of the tests.

DISCUSSION

This study establishes that both the CIE and ELISA systems are reliable diagnostic tests for the diagnosis of *G. lamblia* infections on the basis of established criteria (18). We performed a blinded comparison with a reference standard of diagnosis on stool samples from patients with a spectrum of clinical manifestations. We described the clinical setting and exact methods for the study, the precision of the tests, and variation among observers. The advantage of antigen detection systems is that they provide rapid, sensitive, and reproducible results for large numbers of samples; they may be less labor-intensive, less expensive, and subject to less test-to-test variation than diagnosis of *G. lamblia* infections by microscopy. These assays serve as a useful adjunct to microscopic examination, which is directed to detect a spectrum of enteric infections.

CIE and ELISA antigen detection systems showed results similar to those of microscopic examination in a large, prospective, blinded comparison for the diagnosis of *G. lamblia* in stool specimens. Although cysts are usually identified five times more often than trophozoites in stool by microscopy (4, 21), these assays, which used rabbit sera hyperimmune to two distinct phases of the life cycle of *G. lamblia*, gave comparable results. The recognition of common antigens by these sera, especially those bands of 65 kDa, as first described by Rosoff and Stibbs (17) and Stibbs et al. (19), and those between 30 and 34 kDa, which are also recognized by humans infected with *G. lamblia* (8, 20), may in part explain this similarity. These cross-reactive antigens, which are recognized by the cyst-immune serum, are probably derived from trophozoites within the cysts. This cross-reactivity was not described by another group, perhaps because of differences in immunization technique or in the method of antigen preparation (5). The CIE assay may well detect other protein or nonprotein cyst wall antigens (5, 25).

Antigen detection may be more sensitive, rather than less specific, than the reference standard, microscopic examination (14). The specificity of microscopic diagnosis in these university-affiliated and state laboratories is high in Denver, probably because of the frequency of diagnosis of the parasite and the long-term interest in *G. lamblia* in Colorado. We demonstrated the specificity of the trophozoite-immune serum in vitro by adsorption studies and by the lack of cross-reactivity with other protozoa in clinical samples. The false-positive rate was higher among day-care-center toddlers, among whom carriage rates of *G. lamblia* are high (1, 16; Novotny et al., Abstr. Annu. Conf. Epidemic Intelligence Service, 1986) and who may excrete few organisms (3, 9, 15), than among adults, who are at low risk of infection. False-negative samples were uncommon with both antigen detection methods. The true sensitivities of these tests could be determined best during an outbreak by testing acutely ill persons, with multiple specimens being taken from the same subjects (14), and by using seeding studies.

We conclude that antigen detection systems provide an acceptable and convenient adjunct to microscopic examination for noninvasive method of diagnosis of *G. lamblia* in stool specimens. Further identification of parasite-specific antigens may be useful to standardize and improve the clinical utility of diagnostic methods and to define the natural history of these infections.

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LITERATURE CITED

1. **Black, R. E., A. C. Dykes, S. P. Sinclair, and J. G. Wells.** 1977. Giardiasis in day-care centers: evidence of person-to-person transmission. Pediatrics **60**:486–491.
2. **Craft, J. C., and J. D. Nelson.** 1982. Diagnosis of giardiasis by counterimmunoelectrophoresis of feces. J. Infect. Dis. **145**: 499–504.
3. **Danciger, M., and M. Lopez.** 1975. Number of *Giardia* in feces of infected children. Am. J. Trop. Med. Hyg. **24**:237–242.
4. **Garcia, L. S., T. C. Brewer, and D. A. Bruckner.** 1979. A comparison of the formalin-ether concentration and trichrome-stained smear methods for the recovery and identification of intestinal protozoa. Am. J. Med. Technol. **45**:932–935.
5. **Gillin, F. D., D. S. Reiner, M. J. Gault, H. Douglas, S. Das, A. Wunderlich, and J. F. Sauch.** 1987. Encystation and expression of cyst antigens by *Giardia lamblia* in vitro. Science **235**: 1040–1043.
6. **Gordts, B., W. Hemelhof, K. Van Tilborgh, P. Retore, S. Cadranal, and J. P. Butzler.** 1985. Evaluation of a new method for routine in vitro cultivation of *Giardia lamblia* from human duodenal fluid. J. Clin. Microbiol. **22**:702–704.
7. **Green, E. L., M. A. Miles, and D. C. Warhurst.** 1985. Immunodiagnostic detection of *Giardia* antigen in faeces by a rapid visual enzyme-linked immunosorbent assay. Lancet **ii**:691–693.
8. **Janoff, E. N., P. D. Smith, and M. J. Blaser.** 1988. Acute antibody responses to *Giardia lamblia* are depressed in patients with the acquired immunodeficiency syndrome. J. Infect. Dis. **157**:798–804.
9. **Jokipii, A. M. M., and L. Jokipii.** 1977. Prepatency of giardiasis. Lancet **i**:1095–1097.
10. **Kamath, K. R., and R. Murugasu.** 1974. A comparative study of four methods for detecting *Giardia lamblia* in children with diarrheal disease and malabsorption. Gastroenterology **66**:16–21.
11. **Ma, P., and R. Soave.** 1983. Three-step stool examination for cryptosporidiosis in 10 homosexual men with protracted watery diarrhea. J. Infect. Dis. **147**:824–828.
12. **Mahalanabis, D., T. W. Simpson, M. L. Chakraborty, C. Ganguli, A. K. Bhattacharjee, and K. L. Mukherjee.** 1979. Malabsorption of water-miscible vitamin A in children with giardiasis

and ascariasis. Am. J. Clin. Nutr. **32**:313–318.

13. **Naik, S. R., N. R. Rau, and V. K. Vinayak.** 1978. A comparative evaluation of three stool samples, jejunal aspirate and jejunal mucosal impression smears in the diagnosis of giardiasis. Ann. Trop. Med. Parasitol. **72**:491–492.
14. **Nash, T. E., D. A. Herrington, and M. M. Levine.** 1987. Usefulness of an enzyme-linked immunosorbent assay for detection of *Giardia* antigens in feces. J. Clin. Microbiol. **25**: 1169–1171.
15. **Olveda, R. K., J. S. Andrews, Jr., and E. L. Hewlett.** 1982. Murine giardiasis: localization of trophozoites and small bowel histopathology during the course of infection. Am. J. Trop. Med. Hyg. **31**:60–66.
16. **Pickering, L. K., W. E. Woodward, H. L. DuPont, and P. Sullivan.** 1984. Occurrence of *Giardia lamblia* in children in day-care centers. J. Pediatr. **104**:522–526.
17. **Rosoff, J. D., and H. H. Stibbs.** 1986. Isolation and identification of a *Giardia lamblia* specific stool antigen (GSA 65) useful in coprodiagnosis of giardiasis. J. Clin. Microbiol. **23**:905–910.
18. **Sackett, D. L., R. B. Haynes, and B. Tugwell.** 1985. Clinical epidemiology. Little, Brown & Co., Boston.
19. **Stibbs, H. H., M. Samadpour, and J. F. Manning.** 1988. Enzyme immunoassay for detection of *Giardia lamblia* cyst antigens in Formalin-fixed and unfixed human stool. J. Clin. Microbiol. **26**:1665–1669.
20. **Taylor, G. T., and W. M. Wenman.** 1987. Human immune response to *Giardia lamblia* infection. J. Infect. Dis. **155**: 137–140.
21. **Thornton, S. A., A. H. West, H. L. DuPont, and L. K. Pickering.** 1983. Comparison of methods for identification of *Giardia lamblia*. Am. J. Clin. Pathol. **80**:858–860.
22. **Tijssen, P., and E. Kurstak.** 1984. Highly efficient and simple methods for the preparation of peroxidase and active peroxidase-antibody conjugates for enzyme-immunoassays. Anal. Biochem. **136**:451–457.
23. **Ungar, B. L. P., R. H. Yolken, T. E. Nash, and T. C. Quinn.** 1984. Enzyme-linked immunosorbent assay for the detection of *Giardia lamblia* in fecal specimens. J. Infect. Dis. **149**:90–97.
24. **Vinayak, V. K., F. K. Kum, R. Chandna, K. Venkateswarlu, and S. Mehta.** 1985. Detection of *Giardia lamblia* antigen in feces by counterimmunoelectrophoresis. Pediatr. Infect. Dis. **4**:383–386.
25. **Ward, H. D., J. Alroy, B. I. Lev, G. T. Keusch, and M. E. A. Pereira.** 1985. Identification of chitin as a structural component of *Giardia* cysts. Infect. Immun. **49**:629–634.

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IDENTIFICATION OF CRYPTOSPORIDIUM OOCYSTS BY MONOCLONAL ANTIBODY

SIR,—The protozoan parasite cryptosporidium can cause self-limiting gastroenteritis in otherwise normal individuals or a chronic life-threatening diarrhoea in immunologically compromised individuals, especially those with AIDS.[1] Laboratory diagnosis has relied on the demonstration of oocysts in faeces by modified Ziehl-Neelsen or auramine-phenol staining.[2] One of us (D. P. C.) has also described an indirect fluorescent antibody test using human sera containing anti-cryptosporidium IgA antibodies (HIgA) for confirmatory purposes.[1,2] A monoclonal antibody for diagnostic use has been reported [3,4] but few data on sensitivity and specificity have been presented. We report here a rapid and accurate method using a monoclonal antibody in an indirect immunofluorescent antibody (IFA) test, which seems to be highly specific and more sensitive than conventional techniques. It allows the demonstration of oocysts in hitherto unsuitable samples and their differentiation from bodies that resemble cryptosporidium morphologically.

Balb/c mice were immunised with an enriched oocyst suspension (from the faeces of an infected calf) which had been gamma-irradiated and sonicated before intraperitoneal inoculation at about 10^4 oocysts per inoculum four times over 24 days. Spleen cells taken on the 27th day after the first inoculation were fused with a drug-sensitive tumour cell line (JK-Ag8653), and antibody-secreting hybridomas were identified by an indirect IFA test. A single cell line (MAB C1) secreted antibody of the IgM isotype. At first this was used in an indirect assay, but we have since conjugated the antibody directly with fluorescein isothiocyanate (FITC) and developed a technique with a total assay time of less than 30 min.

Faecal samples were homogenised and concentrated[2] and tested in parallel with conventional staining and HIgA techniques (table). MAB C1 proved to be specific: it reacted with all samples containing cryptosporidium oocysts identified by staining and it did not react with morphologically similar bodies or related parasites. It also seems more sensitive than conventional tests, since cryptosporidium oocysts in follow-up samples from confirmed cases that did not stain by conventional techniques were detectable with MAB C1. The monoclonal antibody was superior to the HIgA because of reagent reproducibility and higher specificity (the HIgA contained antibodies to other organisms).

The epidemiology of cryptosporidiosis is incompletely understood, and this reagent may permit the specific identification of the oocysts and the exclusion of other morphologically similar bodies in environmental samples that are not suitable for testing by conventional staining.

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1. Casemore DP, Sands RL, Curry A. *Cryptosporidium* species a "new" human pathogen. *J Clin Pathol* 1985, **38:** 1321–36.
2. Casemore DP, Armstrong M, Sands RL. Laboratory diagnosis of cryptosporidiosis. *J Clin Pathol* 1985; **38:** 1337–41.
3. Sterling CR, Arrowood MJ. Detection of *Cryptosporidium* sp infections using a direct immunofluorescent assay. *Pediatr Infect Dis* 1986, **5:** S139–42.
4. Sterling CR, Seeger K, Sinclair NA. *Cryptosporidium* as a causative agent of traveller's diarrhoea. *J Infect Dis* 1986; **153:** 380–81.

RESULTS OF COMPARISON OF DIFFERENT METHODS FOR THE DEMONSTRATION OF CRYPTOSPORIDIUM OOCYSTS

	No of specimens	Number of specimens positive by:			
		MZN	AP	HIgA	MAB C1*
Assessment of sensitivity (specimens from individuals with cryptosporidiosis)					
Human faeces (known positive or follow-up samples)	35†	28	29	34	35
Enriched oocyst suspensions	5	5	5	5	5
Calf faeces	2	2	2	2	2
Lamb faeces	2	2	2	2	2
Guineapig faeces	1	1	1	1	1
Mouse faeces (infected by material from a calf)	1	1	1	1	1
Assessment of specificity					
Human faeces (non-cryptosporidiosis)	10	0	0	0	0
Horse faeces (contains CLB and AFB)	5	5	5	0	0
Water filtrates (contains CLB and AFB)	10	10	10	0	0
Eimeria tenella	1	NT	NT	NT	0
Toxoplasma gondii (trophozoites)	1	NT	NT	NT	0

*Higher numbers of oocysts were detected with MAB C1 than with the MZN and AP techniques in 16 samples.
†7 additional follow-up samples were negative with all methods.
MZN = modified Ziehl-Neelsen; AP = auramine-phenol; HIgA = human anti-cryptosporidium IgA antibody; MAB C1 = anti-cryptosporidium monoclonal antibody; AFB = acid-fast bodies; CLB = cryptosporidium-like bodies which were similar but morphologically distinct; NT = not tested.

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