season, this infection episode was analyzed by four different methods: confirmatory statistical analysis, exploratory logistic regression analysis, confidence intervals of incidence density ratios, and risk ratio scoring. The association of infection status with wastewater aerosol exposure and other relevant factors was investigated.

Comparison of crude seroconversion incidence densities indicated that some excess risk of viral infection (risk ratio of 1.5 to 1.8) appeared to be associated with wastewater aerosol exposure. A symmetric risk scoring approach provided evidence of a dose-related stable association (p=0.002) between the infection events in the observed episodes of infection and aerosol exposure. More than the expected number of statistically significant associations of the presence of infection with wastewater aerosol exposure were found in the confirmatory analysis of independent infection episodes using Fisher's exact test. Thus, three different statistical approaches provided similar evidence that the rate of viral infections was slightly higher among members of the study population who had a high degree of aerosol exposure.

In the episode of poliovirus 1 seroconversions in spring 1982, some of the infections were probably caused by wastewater aerosol exposure because a strong association existed and no alternative explanation could be identified. Three distinct risk factors (poliovirus immunization in spring 1982, low polio 1 antibody titer in January 1982, and a high degree of aerosol exposure) were independently associated with the poliovirus 1 seroconversions and each appears to have been responsible for some of the poliovirus 1 infections. Weak evidence of association was found between aerosol exposure and infection by other enteric viruses (specific coxsackie B viruses and echoviruses) which were simultaneously recovered from the wastewater during the summer irrigation season of 1982. However, it could not be determined whether aerosol exposure or identified alternative explanations were the actual risk factor(s) in these enteric viral infections. The association of viral infections with aerosol exposure shows a dose effect, since the study population was exposed to more enteroviruses via the wastewater aerosol in 1982 than in 1983.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Background

Land Application and Potential Infectious Disease Hazards

Land application of wastewater can be an attractive alternative to traditional waste disposal practices. It avoids contamination of surface waters, provides additional waste treatment, returns nutrients to the soil, and reuses the water. The policy of the U.S. Environmental Protection Agency (EPA) is to "press vigorously for publicly-owned treatment works to utilize land treatment processes to reclaim and recycle municipal wastewater." Applicants for federal construction grants (Section 201) must show in their requests that they have considered the application of wastewater to land as an alternative. Financial incentives are provided to encourage land application as stated in the Clean Water Act of 1977. Slow rate application of wastewater to land by spray irrigation has been and continues to be one of the most popular application methods. With EPA encouragement, it is likely that the practice of applying wastewater to land by sprinkler irrigation according to EPA design criteria will become more prevalent as a means of final treatment and disposal.

Along with its considerable benefits, land application of wastewater entails the potential risk of infection from exposure to microorganisms in the wastewater. A variety of agents of human disease, including many overt and potentially pathogenic microorganisms, may survive treatment processes, and thus could theoretically pose a threat. There are various environmental pathways by which these agents in the wastewater and the aerosol produced by its sprinkler application might be introduced and initiate infection in susceptible exposed individuals. Farmers will come in direct contact with the wastewater and its sprayed mist in the course of their work with the irrigation system. Agents in the wastewater aerosol can be transported by the wind and might be inhaled or ingested in exposed food while still viable and infective. Other potential environmental pathways include: 1) ingestion of wastewater-contaminated ground water used as the domestic water supply, 2) dust storms in which wastewater-irrigated surface soils are entrained by strong winds, 3) insect vectors (e.g., flies attracted by the wastewater lagoons), 4) rodents (e.g., feed or food stuffs contaminated by fecal droppings or urine from field mice, infected by wastewater spray, which may be spending the winter in farmhouses and barns), and 5) fomites (e.g., wastewater-contaminated work shoes, clothing, hands, or doorknobs). Once introduced into the local population, the infectious agents might be transmitted by contact between infected and susceptible individuals.

Recent Literature

An Israeli study in 1976 cautioned that the infectious disease hazards associated with irrigation of partially treated wastewater are greater than previously assumed. Existing illness records were analyzed in a retrospective study of enteric diseases among communal agricultural settlements (kibbutzim) in Israel. The incidence rates of enteric illness for kibbutzim utilizing wastewater for spray irrigation were compared with other kibbutzim practicing no form of wastewater irrigation. Two- to four-fold increases in the incidence of shigellosis, salmonellosis, infectious hepatitis, and typhoid fever were reported for the kibbutzim utilizing wastewater, whereas the incidence of other diseases not normally associated with sewage were similar in both groups. A subsequent retrospective study of Israeli kibbutzim in 1983 identified serious deficiencies in the data of the original study, including misclassification of some kibbutzim regarding wastewater reuse, uncertainties about periods of irrigation, and the inadequacy of the communicable disease reports used as the basis for the study. Indeed, the subsequent study failed to find evidence of excess risk associated with wastewater irrigation except in kibbutzim in a "switch" category (i.e., in kibbutzim practicing two consecutive years of wastewater irrigation followed by the same period without irrigation or vice versa). In this category, a significantly increased risk of total enteric disease was noted only for the 0-4 age group during periods of wastewater irrigation.

Two prospective epidemiologic studies were conducted among residents around activated sludge sewage treatment plants near Chicago, Illinois, using the family-based virus watch approach. Both studies included a health watch of participating households that involved health diaries, serology, and clinical specimen isolations. Neither study detected any obvious adverse health effects in residents potentially exposed to wastewater aerosols from aeration basins.
Occupational health effects of wastewater and wastewater aerosols have also been investigated. A study of Muskegon County, Michigan, workers exposed to wastewater spray irrigation failed to show any differences in illness or viral isolation rates between the workers and a control group. Although antibody titers to coxsackievirus B5 were significantly higher in spray irrigation nozzle cleaners, seroconversions were not documented. Likewise, a prospective seroepidemiologic study of municipal sewer and sewage treatment workers and controls in three American metropolitan areas failed to support a significant risk associated with exposure to the wastewater. However, inexperienced workers reported significantly higher rates of gastrointestinal illness, and the level of antibody to certain viruses appeared to be related to level of exposure to wastewater aerosols. In Sweden, an increased incidence of acute febrile illness was found among workers exposed to sludge dust (probably due to endotoxins) and also increased incidence of gastrointestinal symptoms among sewage treatment workers.

None of these studies has investigated the effects on nearby residents' health of sprinkler irrigation of wastewater over a known broad range of wastewater quality. The Lubbock Infection Surveillance Study (LISS) was designed to observe any association of the potential infectious disease effects with exposure to sprayed wastewater.

The Lubbock Land Treatment System (LLTS) Expansion

A major new land treatment system was constructed as a demonstration project to apply wastewater from Lubbock, Texas, by sprinkler irrigation at the Hancock farm near Wilson, Texas, (see Figure 1). The design and operation of this large demonstration project provided for collection of research data under a wide range of quality of the wastewater that was used for irrigation. The first four major irrigation periods after the LLTS expansion commenced operation in February 1982 were monitored. The quality of the applied wastewater was substantially different in each of the four periods. The original spray nozzles directed the wastewater upward, which enhanced the creation and drift of aerosols. Thus, the LISS investigated the risk of wastewater exposure ranging from conditions representative of established guidelines (fecal coliforms <1000 MPN/100 mL) to those which explored the relative safety factor of the guidelines.

The LISS was one of several areas of research which were conducted simultaneously at the land treatment demonstration site. The chemical, biological and physical conditions of the ground water, soils, and crops were characterized prior to and during the wastewater irrigation. The effects of hydraulic, nutrient, and salt mass loading were assessed on the percolate and on the crops and soil.

The Lubbock Infection Surveillance Study (LISS)

The LISS was conducted to monitor infections in the community surrounding the new land treatment demonstration system. This prospective observational study has attempted to determine the association, if any, between the occurrence of infectious diseases in residents and workers and their exposure to the wastewater and aerosols produced by wastewater spray irrigation. The initial two years of operation of the LLTS expansion at the Hancock farm were investigated. LISS involved a 4-year health watch of nearby residents and microbiological monitoring of the wastewater and its aerosol. This site is unique in that a typical rural community with no prior wastewater exposure was challenged by the enteric agents active in a much larger urban community (Lubbock). Persons residing around the Hancock site may have been exposed...
to infectious agents indigenous in the Lubbock population but not circulating in the study area. Thus, many in the study population may have been relatively susceptible to the pathogens in the wastewater. A health watch of the rural community was maintained before, during, and after periods of wastewater spray irrigation. The health watch focused on infections detected serologically and through isolates recovered from routine fecal specimens. To enhance the likelihood of interpreting observed episodes of infection, the likely routes of introduction and transmission were monitored.

Study Objective

The general objective of the LlSS was to identify possible adverse effects on human health from slow rate (sprinkler) land application of wastewater which contained potentially pathogenic microorganisms. More precisely, the objective was to determine the association, if any, between the occurrence of infectious diseases in residents and workers and their exposure to the wastewater and aerosols produced by wastewater spray irrigation. This objective was accomplished by disease surveillance of the study population, by description of the distribution of infections, and principally by evaluation of the incidence of infections for association with exposure.

Study Design

The LlSS was designed to monitor infections and illnesses occurring in the study population and concurrent environmental levels of the infectious agents. Disease surveillance was maintained to protect the population from any obvious untoward effects. However, the study focused on infections and the infecting agents rather than illness in order to obtain greater objectivity, sensitivity, specificity, and etiologic evidence.

All participants were asked to provide blood samples semiannually, usually in June and December. Sera were assayed for antibody titers to specific enteroviruses and other microorganisms known or suspected to be present in the sprayed wastewater. A seroconversion, defined as the four-fold or greater increase in agent-specific antibody titer in simultaneously tested successive sera from one individual, was considered serologic evidence that the individual had been infected by the agent during the time interval between the blood collections. Since mycobacteria were present in the wastewater, tuberculin skin tests were administered annually to give suggestive evidence of a nontuberculosis mycobacterial infection.

An adult from each household and any children under 13 years of age were designated as fecal donors. Each donor, whether well or ill, was asked to submit routine stool specimens for microbiological testing during scheduled weeks which spanned each major irrigation period in 1982 and 1983. A series of three 1-week fecal collection sessions were scheduled before, during, and near the end of each irrigation period to detect infection events occurring in the interim. Clinical bacteriological analyses were performed to isolate overt and opportunistic pathogens. A semiquantitative measurement of growth (as heavy, moderate, light, or very light) was obtained by streaking primary plates by a four-quadrant method. Three categories of bacterial infection events were identified by comparing results from consecutive monthly specimens from an individual. Clinical virological analyses were performed to isolate enteric viruses in the fecal specimens by tissue culture techniques. Electron microscopic examination was performed on about 1/4 of the routine fecal specimens to detect a variety of virus-like particles, many of which are not recoverable by tissue culture techniques. Detection of a specific virus by laboratory cultivation or by electron microscopic examination was considered evidence of a viral infection. Each non-adenovirus viral infection was regarded to be new, unless the same agent had been recovered from the individual in the prior 6 weeks.

Each household was contacted weekly by telephone for a report of any illnesses during the prior week. When a sufficiently recent respiratory or gastrointestinal illness was reported, the ill participant was requested to submit a throat swab or stool specimen to identify the causative agent. Weekly self-reports of illness and appropriate illness specimens were obtained over the entire period of irrigation from January 1982 until October 1983 and over baseline periods corresponding to seasons of heavy irrigation.

The types and densities of potentially pathogenic bacteria and viruses were monitored in the wastewater, wastewater aerosol, and other environmental routes of introduction and transmission. An effort was made to determine the fluctuations in levels of every measurable infectious agent utilized in the health watch. However, the low densities of many agents in environmental samples necessitated reliance on indicator organisms to establish environmental patterns. Wastewater samples of the effluent from the pipeline and reservoirs to be utilized for spray irrigation, and of the Wilson effluent, were obtained and analyzed for indicator bacteria and enteroviruses biweekly to span the major irrigation periods; corresponding baseline samples had been obtained with the same frequency in 1981 and at lesser frequency in 1980 to characterize the effluents. Microbiological screens of indigenous enteric bacteria were conducted on one sample each from the pipeline and the reservoir per irrigation season. The purpose of the routine wastewater samples was to document the presence, prevalence, longitudinal pattern, and passage through the study community of viral and bacterial pathogens possibly introduced by the wastewater. Extensive aerosol sampling was conducted to characterize the aerosol density of indicator microorganisms produced by the spray irrigation of both pipeline and reservoir wastewater. Virus runs were also conducted to measure the density and diversity of enteroviruses in aerosols emanating from the sprinkler rigs. Drinking water, houseflies, and dust storms also were evaluated as other means of introducing microorganisms into the study population.

An aerosol exposure index (AEI) was devised to measure the degree of a participant’s cumulative exposure to microorganisms in the wastewater aerosol, relative to all other study participants during a given irrigation period. When a number of similar infection events were observed either serologically or microbiologically in the study population within a time interval corresponding to an irrigation period, this infection episode was statistically analyzed for association with wastewater aerosol exposure using AEI. Infection incidence rates were compared among exposure subgroups and with baseline rates to determine the relative risk of infection.

Conclusions

1. The LlSS employed an epidemiologic analytic prospective cohort study design which was quite appropriate to measure the strength of association between exposure to the wastewater used for irrigation and the development of new infections. The results from the isolation and serology procedures used to detect infections appear to be adequate. These detection methods were sufficiently sensitive and specific to observe many episodes of infection in the study
population in which the etiologic agent was identified. The size of the population was sufficient to analyze the distribution of observed infections for possible association with exposure to wastewater irrigation and to control for extraneous variables via logistic regression analysis. However, the small population size led to instability of the association. The significance of the study findings have not been limited to a great extent by such major confounding factors as age, gender, antibody level, head of household education, and time spent in Lubbock.

2. The quality of the wastewater to which the study population was exposed was highly variable during the study. During the initial spring 1982 irrigation period, the quality of the irrigation wastewater approached that of a low quality primary effluent, as determined by physical and chemical analyses. While the quality of the irrigation wastewater was greatly improved in 1983, its fecal coliform concentration still exceeded the EPA guideline for controlled trol for extraneous variables via for possible association with exposure the distribution of observed infections than background levels in ambient air microorganisms via the wastewater aerosol was greater in study population received to most the small population size led to in-stability of the association. The significance of the study findings have not been limited to a great extent by such major confounding factors as age, gender, antibody level, head of household education, and time spent in Lubbock.

3. Spray irrigation of wastewater obtained via pipeline directly from the Lubbock SeWRP was a more substantial source of aerosolized microorganisms than spray irrigation of wastewater stored in reservoirs. Enteroviruses were consistently recovered in the aerosol at 44 to 60 m downwind of irrigation with pipeline wastewater.

4. Microorganism levels in air downwind of spray rigs using pipeline wastewater were significantly higher than upwind levels: fecal streptococci levels to at least 300 m downwind, and levels of fecal coliforms, mycobacteria and coliphage to at least 200 m downwind. Levels downwind were also significantly higher than background levels in ambient air outside of participants' homes: fecal coliform levels to beyond 400 m downwind, mycobacteria and coliphage levels to at least 300 m and fecal streptococci levels to at least 200 m.

5. The exposure which most of the study population received to most microorganisms via the wastewater aerosol was greater in 1982 than in 1983. The cumulative enterovirus dose received from aerosol exposure at a given distance downwind in summer 1982 was estimated to be at least an order of magnitude greater than in any other irrigation period.

6. Individuals in the high (AEI>3) and low (AEI<3) exposure groups were generally well balanced with regard to infection risk factors, including age, gender and previous antibody titer. The high exposure fecal donors ate food prepared by a local restaurant very significantly more often, made greater use of evaporative coolers for air conditioning, and had more farmers as head of household.

7. The lack of a strong, stable association of clinical illness episodes with the level of exposure to irrigation wastewater indicates that wastewater spray irrigation did not produce obvious disease during the study period. However, the participants in the high exposure level (AEI>5) reported a slight excess crude incidence density of total acute illness shortly after the onset of wastewater irrigation, both in spring 1982 and in summer 1982, the seasons of initial and heaviest microbial exposure, respectively. The extent to which this reflects actual illness versus possible reporting bias by high exposure participants cannot be ascertained.

8. The occurrence of enteric Gram-negative bacteria (EGNB) at moderate and heavy levels in the throats of both healthy and ill study participants was frequent and widespread between July 19 and October 12, 1982. The household environment was strongly associated with the continuing EGNB throat infections of one household. Among the ill throat swab donors, use of an evaporative cooler for home air conditioning was associated with the EGNB throat infections.

9. Some excess risk of viral infection (risk ratio of 1.5 to 1.8) was associated with wastewater aerosol exposure, based on comparison of crude seroconversion incidence densities by aerosol exposure level and by irrigation vs. baseline period.

10. A symmetric risk ratio score approach provided evidence of a stable and dose-related association between infection events and wastewater aerosol exposure in the infection episodes observed by the LISS.

11. Some infection episodes appear to have been related to wastewater aerosol exposure, because more statistically significant associations than expected were found in the confirmatory analysis of independent infection episodes using a one-sided Fisher's exact test. Some imbalances in the two populations may provide alternate explanations for the excess associations. On the other hand, the number of detected increases in incidence rates associated with the wastewater irrigation may be underestimated, considering the relatively modest power of the tests to detect small differences.

12. An exploratory logistic regression analysis found significant (p<0.05) associations between presence of infection and degree of aerosol exposure while controlling for the effects of extraneous variables in four infection episodes. More supporting evidence was found for the wastewater aerosol route of exposure than for direct contact with wastewater or spending time in the irrigation environment on the Hancock farm.

13. Eight specific infection episodes displayed good or marginally consistent evidence of association with wastewater aerosol exposure.

a. Two of these episodes were probably unrelated to wastewater exposure because a more plausible alternative explanation was identified:
   - Episode of Klebsiella infections in summer 1983
     — alternative: eating at a local restaurant
   - Spurious control episode of echovirus 9 seroconversions in the baseline period
     — alternative: within household spread

b. The evidence is inconclusive in five episodes because both aerosol exposure and the identified alternative explanation(s) are plausible risk factors:
   - Episode of clinical viral isolates excluding adenoviruses and immunization-associated polioviruses in summer 1982
     — alternative: eating at a local restaurant
   - Episode of echovirus 11 seroconversions in 1982
     — alternatives:
       • contaminated drinking water
       • Caucasian, large household
   - Episode of seroconversions to viruses isolated from wastewater in summer 1982
14. Despite the efforts to obtain a random sample, the study participants during the irrigation periods were essentially volunteers who were not representative of the entire population of the study area. Furthermore, the frequency of patronizing local restaurants and the use of evaporative coolers were factors that were largely confounded with wastewater aerosol exposure. For these reasons, the LISS findings cannot easily be generalized to other sites.

15. In summary, a general association existed between exposure to irrigation wastewater and new infections. A viral dose-response relationship was observed over the four irrigation seasons, since the aerosol exposure-associated episodes of viral infection occurred primarily in 1982 during the irrigation seasons of greater enterovirus aerosol exposure. Some poliovirus 1 seroconversions during the spring of 1982 were probably related to wastewater aerosol exposure. However, even during 1982, the strength of association remained weak and frequently was not stable.

16. Wastewater of poor quality comprised much of the irrigation water in 1982. Of the many infection episodes observed in the study population, few appear to have been associated with wastewater aerosol exposure, and none resulted in serious illness.

Recommendations

1. To minimize exposure, it would be prudent to use wastewater from the reservoirs at the Hancock farm for irrigation (or to apply equivalent treatment measures), rather than irrigating directly from the pipeline.

2. Poliovirus serology should be performed on archived sera from June 1982 through October 1983 to identify poliovirus seroconversions in the study population spanning the summer 1982 and the 1983 irrigation periods. Any observed poliovirus infection episodes should be fully analyzed by the inferential methods employed in the LISS. Since summer 1982 and possibly summer 1983 appear to have been seasons of higher poliovirus aerosol exposure than spring 1982 was, these data would confirm or dispute the probable relationship of poliovirus 1 seroconversions to wastewater aerosol exposure which was observed in spring 1982.

3. Serological testing of archived sera is recommended for selected enteroviruses and rotavirus to observe and analyze additional infection episodes in order to clarify the apparent dose-response relationship with wastewater aerosol exposure detected in the LISS.

4. An exposure assessment should be performed to estimate the range of cumulative organism exposure dosages that applied to the LISS infection episodes and other situations in which reasonable evidence of association with wastewater irrigation was obtained. A dosage to the infectious agent should be estimated for each infected individual and the dosage range of the high exposure level of participants should be approximated.

5. An improved model of microbiological dispersion should be developed based on the LISS aerosol sampling data. The LISS data provide a much better basis for model development than the data bases previously employed. The model would permit the determination of the estimated range of microorganism exposure dosages at considerable distances downwind (i.e., 400-800 m) from any spray irrigation source of wastewater aerosols.

6. If recommendation 1 is not implemented, a limited program of wastewater and aerosol sampling should be conducted at the Hancock farm to determine densities of enteroviruses and indicator bacteria in wastewater and downwind air and to reevaluate aerosolization efficiency for the current treatment process and mode of operation. "Pulsed breakpoint chlorination" of pipeline wastewater and installation of proper spray nozzles to reduce aerosol formation and drift are two major changes in irrigation practices at the Hancock farm.
farm since 1983. The sampling program would permit determination of where the current irrigation practices fit into the seasonal dose-effect gradient found in the LISS.

7. It is recommended that analyses of existing LISS data be performed as pilot studies to investigate whether clinically and serologically detected infections and self-reported illness were associated with several apparent environmental sources of infection identified in the LISS.
   a. Evaluate bacterial contamination of wells that served as sources of household drinking water.
   b. Evaluate patronage of local restaurants in this rural community to help to address the extent to which food prepared for public consumption may be a source of apparent infections and minor acute illness.
   c. Evaluate the use of evaporative coolers for air conditioning as a source of bacterial infections and illness, especially when bacterial contamination of water supplies is quite widespread.

8. Certain additional data analyses are recommended to facilitate proper interpretation of the LISS results:
   a. Calculate incidence density ratios and their confidence intervals for clinical agents, as was done for serologic agents and self-reported illness, in order to balance the procedure for selection of infection episodes with good and marginal evidence of association with aerosol exposure.
   b. Investigate the need to control by logistic regression analysis for the effects on infection status of three additional factors which were partially confounded with wastewater aerosol exposure: evaporative cooler use prior to 1983, rural versus Wilson location, and children in the household.
   c. Conduct a stratified analysis of serologic and illness incidence densities to control for major potential risk factors, such as age, gender, previous antibody titer, occupation and education of head of household, restaurant patronage, and dwelling location. These analyses would clarify interpretation of apparent associations with aerosol exposure of seroconversions and self-reported illness which were based on test-based confidence intervals of crude incidence density ratios.
   d. Determine if there is evidence of association of infections with residential aerosol exposure when the individuals with occupational exposure to wastewater irrigation are excluded from the study population.

The LISS was conducted by Southwest Research Institute (SwRI), the University of Illinois (UI), the University of Texas at San Antonio (UTSA) and the University of Texas at Austin (UTA). The full report was submitted in fulfillment of CR 807501 and S806204 by SwRI under primary sponsorship of the U.S. Environmental Protection Agency. The full report covers field activities performed from May 1, 1980, to October 31, 1983; work was completed as of June 30, 1985.

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The complete report, entitled “The Lubbock Land Treatment System Research and Demonstration Project: Volume IV. Lubbock Infection Surveillance Study (LISS),” (Order No. PB 86-173 622/AS; Cost: $46.95, subject to change) will be available only from:

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